Pseudalert®/Quanti-Tray® for the enumeration of Pseudomonas aeruginosa in human consumption waters and pool waters

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Summary report

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Preamble

***Studied method**:*
Pseudalert for the enumeration of *Pseudomonas aeruginosa*

***Validation standard**:*
Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013).

***Reference method**♦ :*

- NF EN ISO 16266: 2008 “Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration

Scope:
Human consumption waters and pool waters

***Certification body**:*
AFNOR Certification (http://nf-validation.afnor.org/)

♦ Analyses performed according to the COFRAC accreditation

1 Introduction

The **Pseudalert ®/ Quanti-tray®** method was validated in March 2016 (certificate number IDX 33/05-03/16). The certificate was renewed in March 2020 according to the validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013).

In 2024, IDEXX wished to renewal this method. Following the revision of standard **ISO 8199:2018** (Water quality - General requirements and guidance for microbiological examinations by culture), additional data have been produced for the relative accuracy study for the "pool water" category in order to meet the requirements of the validation protocol. For the category “human drinking water”, only statical analysis have been performed by removing data superior to 80 CFU/test portion (100 mL or 250 mL) with the reference method.

1.1 History of validation

The history of validation was summarized in the table below:

Method	Approval	Type of certification	Comments	Expert laboratory	Protocol of validation
Pseudalert ®/Quanti-Tray®	11/03/2016	Initial Validation		ISHA	Rev. 2 (2013)
	March 2020	Renewal 1	No change	AdGène Laboratoire	Rev. 2 (2013)
	February 2024	Renewal 2	Production of new data for the pool water category & Reinterpretation of the relative accuracy results due to the uptade of the NF EN ISO 8199:2018	Normec Abiolab AdGène	Rev 2 (2013)

There were no modifications of the alternative method since the initial validation.

2 Method Protocol

2.1 Alternative method

The Pseudalert test detects *Pseudomonas aeruginosa* in human consumption waters and pool waters. The test is based on a bacterial enzyme detection technology that signals the presence of *Pseudomonas aeruginosa* through the hydrolysis of a substrate present in the Pseudalert reagent. *Pseudomonas aeruginosa* cells rapidly grow and reproduce using the rich supply of amino acids, vitamins and other nutrients present in the Pseudalert reagent. Actively growing strains of *Pseudomonas aeruginosa* have an enzyme that cleaves the substrate to produce a blue fluorescence under ultraviolet (UV) light. Pseudalert detects *Pseudomonas aeruginosa* at 1 MPN in either 100 mL or 250 mL samples within 24 hours.

The alternative method protocol in appendix 2.

2.2 Reference method

The alternative method was compared to the standard NF EN ISO 16266: 2008 “Detection and enumeration of *Pseudomonas aeruginosa* – Method by membrane filtration”.

The protocol of the reference method is presented in appendix 1.

3 Results

3.1 Methods comparison study

The data come from of the initial validation (2016 - ISHA Laboratory)

The goal of the comparative study is to evaluate the performances of the alternative method against the reference method. The following parameters were studied:

- the relative accuracy,
- the linearity,
- the quantification limits,
- the selectivity,
- the practicability.

3.1.1 Relative accuracy

The relative accuracy is the degree of correspondence between the response obtained by the reference method and the response obtained by the alternative method on the same samples.

- **Number and nature of samples**

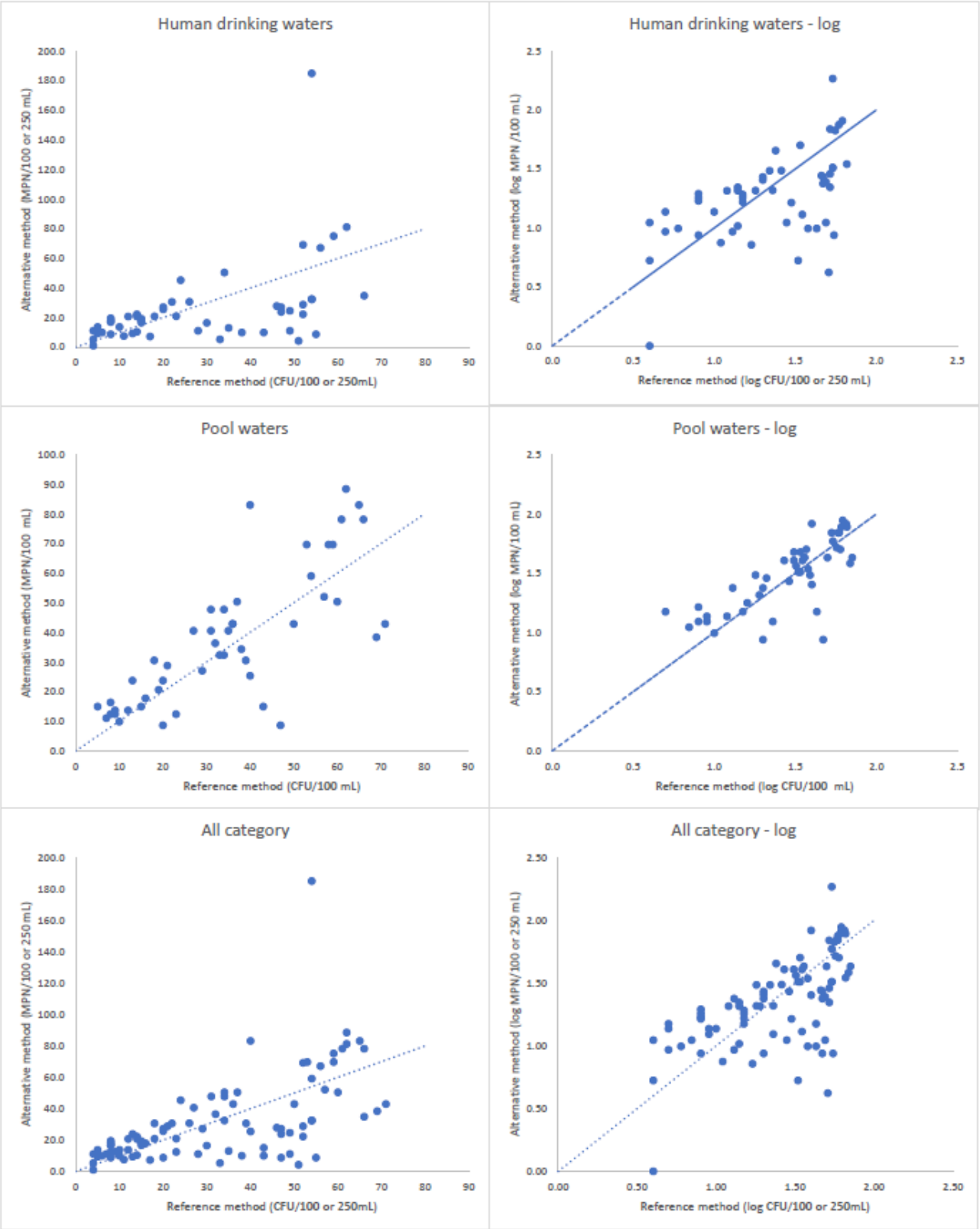
Two categories of waters were tested in duplicate with the alternative method and the reference method.
Samples analyzed are presented in table 1.

Table 1 : number and nature of samples analyzed

Category	Water type	Samples analyzed	Samples exploited
Human consumption waters	Tap water / Fountain water	18	13
	Bottled water	34	12
	Boreholes water	4	1
	Total	**56**	**26**
Pool waters		50	24
TOTAL		**106**	**50**

A total of 106 samples was analyzed and 50 were exploited. Samples that were not retained in the statistical analysis correspond to samples for which enumerations were inferior to 4 CFU/test portion and superior to 80 CFU/test portion (100 mL or 250 mL) with the reference method or superior to the limit of detection for at least one of the replicates of the two methods.
All samples were artificially contaminated. The contamination levels used cover all the measuring range of the alternative method. The stress applied and the strains used are presented in appendix 3.

Figure 1 : two-dimensional graph for relative accuracy in (CFU or MPN / test portion) (black line: y=x)



Raw results and statistical calculations are summarized in tables 2 to 5 and in appendix 4.

Table 2: statistical data (CFU or MPN / test portion) for the enumeration in drinking waters and pool waters

Category	Rob R	Regression used	T_{crit}	a	t(a)	b	t(b)	$-T_{crit}<t(a)<T_{crit}$	$-T_{crit}<t(b)<T_{crit}$
Human drinking waters	0.910	GMFR	2.056	-17.269	0.358	1.491	0.364	Oui	Oui
Pools waters	1.633	GMFR	2.064	-3.272	0.110	1.176	0.233	Oui	Oui
All categories	1.188	GMFR	2.009	-16;547	0.477	1.362	0.386	Oui	Oui

Table 3: statistical data (log CFU or log MPN / test portion) for the enumeration in drinking waters and pool waters

Category	Rob R	Regression used	T_{crit}	a	t(a)	b	t(b)	$-T_{crit}<t(a)<T_{crit}$	$-T_{crit}<t(b)<T_{crit}$
Human drinking waters	1.666	GMFR	2.056	-0.093	0.073	1.012	0.013	Oui	Oui
Pools waters	1.609	GMFR	2.064	0.106	0.127	0.949	0.089	Oui	Oui
All categories	1.376	GMFR	2.009	-0.272	0.146	1.028	0.022	Oui	Oui

Table 4: bias and repeatability of the two methods (CFU or MPN / test portion)

Category	Bias (D)		Standard deviation of repeatability robust		Contamination area
	Mean	Median	Reference method	Alternative method	
Human drinking waters	-3.025	0.950	2.605	14.361	[4 ; 185]
Pools waters	2.671	3.025	3.653	4.642	[5 ; 89]
All categories	-0.291	2.300	3.098	10.777	[4 ; 185]

Table 5: bias and repeatability of the two methods (log CFU or MPN / test portion)

Category	Bias (D)		Standard deviation of repeatability robust		Contamination area
	Mean	Median	Reference method	Alternative method	
Human drinking waters	-0.077	0.028	0.089	0.112	[0.602 ; 2.267]
Pools waters	0.033	0.060	0.071	0.067	[0.699 ; 1.949]
All categories	-0.024	0.060	0.081	0.095	[0.602 ; 2.267]

The equations for the regression line and the correlation coefficient are as follows:

Category	Raw data		Log data	
	Regression line	Correlation coefficient (r)	Regression line	Correlation coefficient (r)
Human drinking waters	Alt. = 1.491 Ref. – 17.269	0.494	log Alt. = 1.012 log Ref. – 0.093	0.541
Pool waters	Alt. = 1.176 Ref. – 3.272	0.775	log Alt. = 0.949 log Ref.– 0.106	0.790
All categories	Alt. = 1.362 Ref. – 16.547	0.620	log Alt. = 1.028 log Ref. – 0.272	0.640

- **Conclusion**

The bias between the alternative method and the reference method is low and is respectively:

- 0.028 log CFU/250mL for the human drinking water category
- 0.060 log CFU/100mL for the pool water category
- 0.060 log CFU/ 100 or 250 mL for all categories

The regression line equations are respectively:

- log (Alt) = 1.012log (Ref) - 0.093 for the human drinking water category
- log (Alt) = 0.949log (Ref) - 0.106 for the pool water category
- log (Alt) = 1.028log (Ref) - 0.272 for all categories

The standard deviations of repeatability between the alternative and reference methods are very close.

Statistical tests validate the accuracy of the alternative method by validating the hypothesis that a is equal to 0 and b is equal to 1 for a risk α=0.05 for the water for human drinking and pool water categories.

3.1.2 Linearity

The linearity is the ability of the method when used with a given matrix to give results that are in proportion to the amount of analyte present in the sample, that is an increase in analyte corresponds to a linear or proportional increase in results.

- **Contamination levels**

The couples matrix / strain are presented in table 6.

For these couples, six levels of contamination were tested in duplicate by the reference method and the alternative method.

Table 6 : couple matrix – strain analyzed

Strain	Matrix	Contamination level (CFU/100 mL)
Pseudomonas aeruginosa	Pool water	10 – 30 – 50 - 100
Pseudomonas aeruginosa	Spring water	10 - 20 -30 – 50 - 100

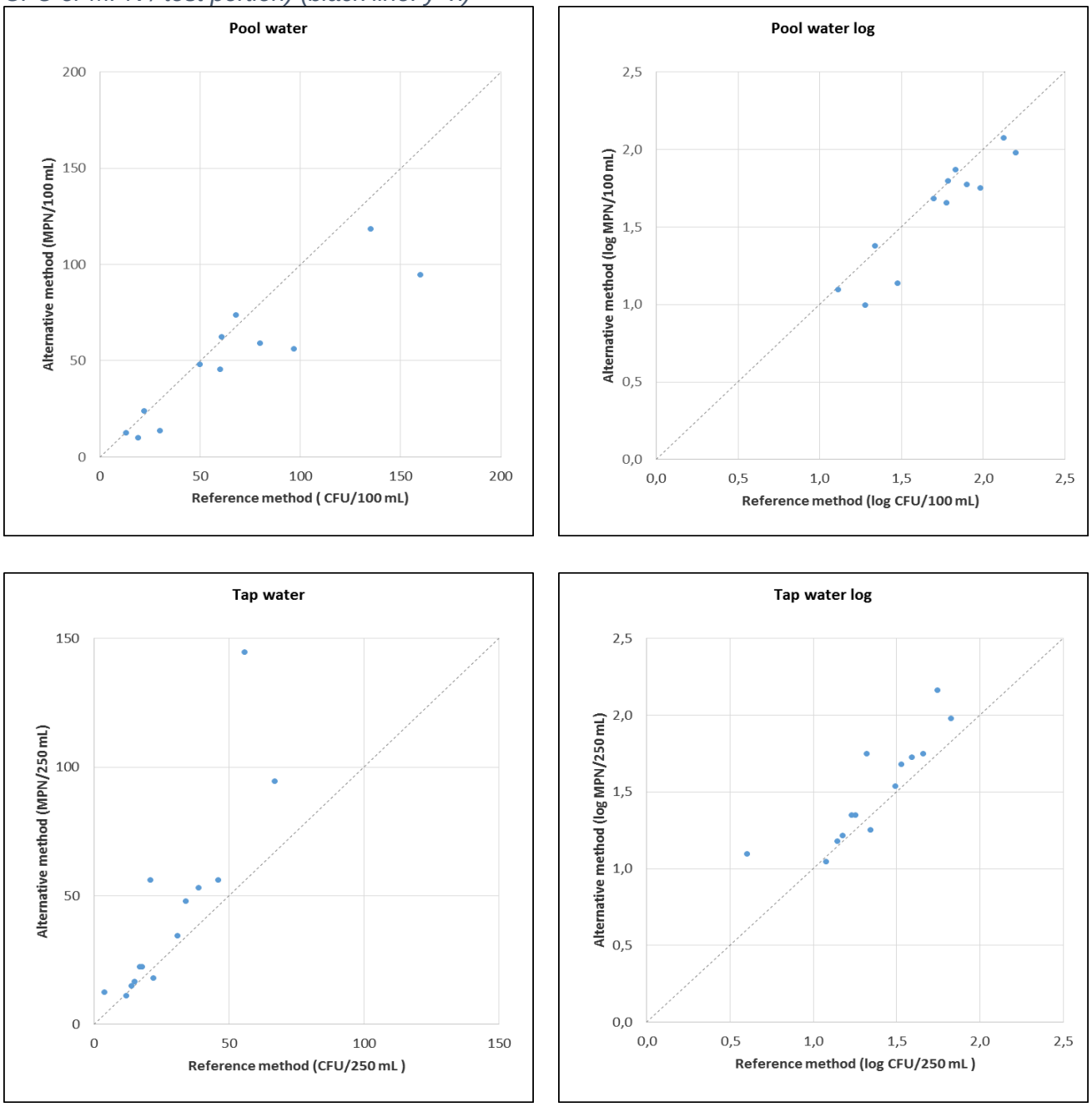
- **Raw results**

Raw results and statistical calculations are summarized in appendix 5.

Graphs of figure 2 show the values of each sample obtained by the alternative method and the reference method. The y-axis is reserved for the alternative method and the x-axis for the reference method.

The representation of a line of equation 'y = x' figures dashed on the graphs.

Figure 2: two-dimensional graphs for linearity of the pool water (CFU or MPN / test portion and log CFU or MPN / test portion) (black line: y=x)



- **<u>Statistical exploitation</u>**

Statistical interpretations are carried out in accordance with the requirements of standard NF ISO 16140 (see table 7).

The linear regression method is chosen over the value of the robustness of the ratio R of overall repeatability standard deviation:
- If Rob.R > 2, linear regression by least-squares (OLS 1) is used with the x-axis for the reference method,
- if Rob.R < 0.5, a linear regression by least-squares (OLS 2) is used with the x-axis for the alternative method,
- If 0.5 < Rob.R < 2, orthogonal regression (GMFR) is used with the x-axis to the reference method.

Table 7: statistical data of the couple matrix – strain analyzed

Matrix	Data	Rob. R	Regression used	F critical	Rob. F	P (Rob.F)	Correlation coefficient (r)	Regression line
Pool water	Raw	1.300	GMFR	4.53	1.145	0.419	0.969	0.733 Ref + 2.875
	Log	1.257	GMFR	4.53	0.837	0.548	0.981	1.050 log Ref – 0.198
Spring water	Raw	0.530	GMFR	3.97	9.201	0.006	0.980	2.071 Ref – 15.492
	log	0.857	GMFR	3.97	5.433	0.023	0.943	1.078 log Ref + 0.040

The relationship between the 2 methods is not linear:
- if Rob.F > critical F or,
- if P (Rob.F) < α (= 0,05).

- **Conclusion**

For the pool water matrix, the relationship between the two methods is linear regardless of the data used (raw data or log data).

For the spring water matrix, the relationship between the two methods is not linear with the raw data and the log data. However, the correlation coefficients of the regression lines are satisfactory.

The linearity of the alternative method is satisfactory.

3.1.3 Detection and quantification limits

The detection and quantification limits are checked in accordance with the standard EN ISO 16140. Three parameters are determined.

Here are their ISO 16140 definitions:

- the critical level (LC) is the smallest amount which can be detected (not null), but not quantified as an exact value. Below this value, it cannot be sure that the true value is not null. At this level, the false negatives probability β is 50 % (β is the second type of statistical error).

- the detection limit (LOD) is higher than the critical level, because it involves a power, the probability 1 - β, which has to be well over 50 %, for example 95 %.

- the quantification limit (LOQ) is the smallest amount of analyte, (that is the lowest actual number of organisms), which can be measured and quantified with defined precision and accuracy under the experimental conditions by the method under validation.

- **Protocol**

Detection and quantification limits were determined by analyzing a pure culture of a *Pseudomonas aeruginosa* strain PSE.1.13, isolated from water, by the alternative method.
Six levels of contamination, with six repetitions for each level, have been studied in sterilized water.

- **Results**

Raw results are presented in appendix 6 and the summary in the following tables.

Table 8: data (s0 and x0) for the enumeration of P. aeruginosa (underlined: the reference level)

Level (MPN/100mL)	Number of positive samples	Standard deviation (s0)	Bias (Xo)
0.000	0	0.000	0.000
0.530	0	0.000	0.000
0.600	0	0.000	0.000
1.500	1	0.816	0.000
2.100	5	1.451	1.000
2.800	6	1.058	2.000

Table 9: LC, LOD and LOQ values of the alternative method for the enumeration of Pseudomonas aeruginosa

Parameter	Formulas	Value obtained
Critical level	$1.65\ s_0 + x_0$	3.39
Detection limit	$3.3\ s_0 + x_0$	5.79
Quantification limit	$10\ s_0 + x_0$	15.51

- **Conclusion**

The limits of detection and the limits of quantification of the alternative method are satisfactory.

3.1.4 Selectivity

The selectivity of the alternative method is evaluated by its inclusivity and its exclusivity.
Inclusivity is the ability of the alternative method to detect the target analyte from a wide range of strains.
Exclusivity is the lack of interference by a relevant range of non-target strains with the alternative method.

- **Protocols**

Twenty four target strains and thirty three non-target strains (from national, international and internal collections) were analyzed. The tests were conducted according to the protocol of the alternative method.

In exclusivity, strains 28 to 32 were isolated from water samples analyzed on CN agar. They produced non-typical colonies on this media.

The contamination levels used for inclusivity were between 30 and 100 CFU / 100mL and for exclusivity 10^3 to 10^5 times higher than the level of detection of the alternative method (approximately 10^4 CFU / 100 mL).

- **Results**

The results are presented in appendix 7.

Twenty four strains of *Pseudomonas aeruginosa* are detected by the alternative method.
The strain PSE.1.12 was initially tested with the inclusivity protocol. This strain was not detected by the alternative method (even with a high contamination level) whereas it was detected by the reference method (and confirmed by all the confirmation tests of the reference method). This strain has been tested in inclusivity because it was initially not detected in some samples for the determination of the relative accuracy.
An identification of this strain by molecular biology showed that the strain was a *Pseudomonas monteilii*. This strain was consequently moved to the exclusivity list. All the twenty four strains of the inclusivity list were detected.

No non-target strain showed cross-reaction with the alternative method.

- **Conclusion**

The selectivity of the method is satisfactory.

- **Practicability**

The practicability is studied by filling in the 13 criteria defined by the Technical Board.

- Procedure for conditioning the elements of the method

Pseudalert reagent is packaged in sealed individual capsules. The Quanti-Tray are conditioned by 10 in sterile plastic bags.

- Reagent volume

Several formats are available (20 tests, 100 tests or 200 tests).

- Conditions of storage of the elements (expiry date for unopened products)

Pseudalert reagent storage temperature is between 2 and 25 ° C.
The storage temperature of the Quanti - Tray is between 4 and 30 ° C.

Products have a 12 months DLC.

- Modalities of use after the first use (expiry dates for use)

Each Quanti-Tray and each Pseudalert reagent capsule serves a unique analysis and must not be re-used.

- Specific equipment or premises required

A Quanti-Tray Sealer model 2X or a Quanti-Tray Sealer PLUS is required.

- Reagents ready-to-use or to be reconstituted

There is no reagent to restore.

- Period required to train an operator not initiated into the method

The use of the method Pseudalert reagent / Quanti-Tray requires no specific training. The duration of training is estimated at 1 hour.

- Real-time handling and flexibility of the method depending on the number of samples to be analyzed.

The duration of an analysis by the method NF EN ISO 16266 is about 1.5 min using disposable filtration units of 3.5 min using non-disposable filtration units. The duration of use of the method Pseudalert is about 4 min (time including: dissolution of the Pseudalert reagent waiting time and the time for sealing the Quanti-Tray).
The alternative method doesn't require any confirmation step or using toxic reagent.

- Time required for obtaining the results

Time-to-result for the method Pseudalert is 24 - 28 hours.
Time-to-result for the method EN ISO 16266 is 48 hours or 4 days if confirmations are required.

- Operator qualification type

The qualification of the operator is similar to the qualification needed for the reference method.

- Phases shared with the reference method

None.

- Means or traceability of the analysis results for the user

No traceability procedure is proposed. The laboratory shall use its internal procedures.

- Obligation to maintain specific apparatus for the user

None.

3.1.5 Conclusion

The linearity and the relative accuracy of the method Pseudalert for the enumeration of *Pseudomonas aeruginosa* in drinking waters and pool waters are satisfactory.
Bias between the two methods is acceptable. The limits of detection and quantification of the method are satisfactory.
The method Pseudalert for the enumeration of *Pseudomonas aeruginosa* is specific and selective.
Results are obtained in 24 to 28 hours with the alternative method against 48 to 96 hours with the reference method.

3.2 Interlaboratory study

The goal of the interlaboratory study is to determine the performances of the alternative method in several laboratories in the real conditions of the “routine” application of the method.

- **Interlaboratory study implementation**

The data come from of the initial validation (2016 - ISHA Laboratory)

- **Participating laboratories**

The interlaboratory study was realized by the expert laboratory and fourteen participating laboratories.

Each laboratory received the instructions relative to the organization of the study a week before its beginning.

- **Matrix and strain**

A tap water was used as test matrix. It was contaminated with a strain of *Pseudomonas aeruginosa* (PSE.1.4) isolated from a fountain water.
The absence of *Pseudomonas aeruginosa* in this matrix before the contamination was checked using the reference method.

- **Stability of the strain in the test matrix**

The stability of the strain in the matrix was evaluated for 3 days at 5±3°C. Results of the enumerations are presented in table 10.

Table 10: results of the enumerations in CFU/100 mL of the strain Pseudomonas aeruginosa in tap water for 3 days at 5±3°C

	Level 1	Level 2	Level 3
D0	169	256	277
D3	86	128	150

A diminution of the concentration of the tested strain is observed from day 0 to day 3 at 5±3°C in the matrix.

- **Samples preparation and spiking**

The matrix was inoculated with the target strain suspension to obtain four contamination levels:

- level 0 : 0 CFU/100 mL,
- level 1 : from 10 to 20 CFU/100 mL,
- level 2 : from 50 to 70 CFU/100 mL,
- level 3 : from 100 to 120 CFU/100 mL.

The matrix was distributed at 250 mL in sterile bottles. Every bottle was individually spiked and homogenized. Eight samples per laboratory were prepared (2 samples per contamination level). Each laboratory received 8 samples to analyze, 1 sample to quantify culturable microorganisms and 1 water sample containing a temperature probe.

The results of the enumerations of culturable microorganisms, the target levels and the real levels of contamination are presented in table 11.

Table 11: target level, real level and endogenous flora of the matrix

Level	Culturable microorganisms (CFU/mL)		*Pseudomonas aeruginosa*.PSE.1.4 (CFU /100 mL)	
	22°C	36°C	Target level	Real level at D0
0	8	2	/	/
1			40	40
2			100	60
3			150	80

- **Samples labeling**

The labeling of the vials was realized as follows: a code to identify the laboratory: from A to N and a code to identify each sample, only known by the expert laboratory (cf. table 12).

The samples and the temperature control vials (water sample with a temperature probe) were stored at 5°C before shipping.

Table 12: sample code by contamination level

Level (CFU / 100 mL)	Sample code
0	2 / 8
10 to 20	4 / 7
50 to 70	5 / 6
100 to 120	1 / 3

- **Samples shipping, reception and analysis**

The samples were shipped in a coolbox the 8th of February 2016.
The coolboxes were received in 24 hours for eleven laboratories and in 48 hours for three laboratories.
The control temperature was recorded upon receipt of the package and the temperature probe sent to the expert laboratory.
The samples were analyzed the 10th of February. The expert laboratory concurrently analyzed a set of samples under the same conditions with both methods.

Analyses were thus realized by fifteen laboratories.

3.2.1 Results

- **Temperature and state of the samples at reception**

The temperature readings at reception, the state of the samples and probes data are shown in table 13.

Table 13: temperature and state of the samples upon reception

Laboratory	Temperature	State of samples	Probe temperature	
			Mean	Maximum
A	7.8 °C	Correct	2.69 °C	4.98 °C
B	7.2 °C	Correct	2.69 °C	4.58 °C
C	**8.5 °C**	Correct	2.14 °C	4.51 °C
D	5.9 °C	Correct	2.63 °C	3.98 °C
E	5.1 °C	Correct	1.62 °C	5.50 °C
F	1.8 °C	Correct	1.63 °C	3.50 °C
G	**9.7 °C**	Correct	2.95 °C	6.06 °C
H	6.4 °C	Correct	3.72 °C	6.01 °C
I	0.9 °C	Correct	2.39 °C	5.00 °C
J	7.8 °C	Correct	2.39 °C	4.00 °C
K	7.4 °C	Correct	2.38 °C	6.00 °C
L	6.2 °C	Correct	2.48 °C	6.00 °C
M	4.2 °C	Correct	2.49 °C	6.99 °C
N	4.3 °C	Correct	2.63 °C	6.07 °C

Temperatures are correct for 14 laboratories. Laboratories C and G showed temperatures superior to 8°C but the analyses of the thermal profiles of the probes showed that the shipping of the samples were realized at a correct temperature, with means between 2.1°C and 3.0°C.

- **Results from expert laboratory and participating laboratories**

The overall results are presented in table 14. Detailed results are provided in appendix 8.
For level 0, the results for one repetition of the alternative method for the laboratory C were superior at 200.5 MPN / 100 mL but all the results of the reference method were inferior to 1 CFU/100 mL

All results of the alternative method for the thirteen other laboratories were inferior to 1 MPN/100 mL.

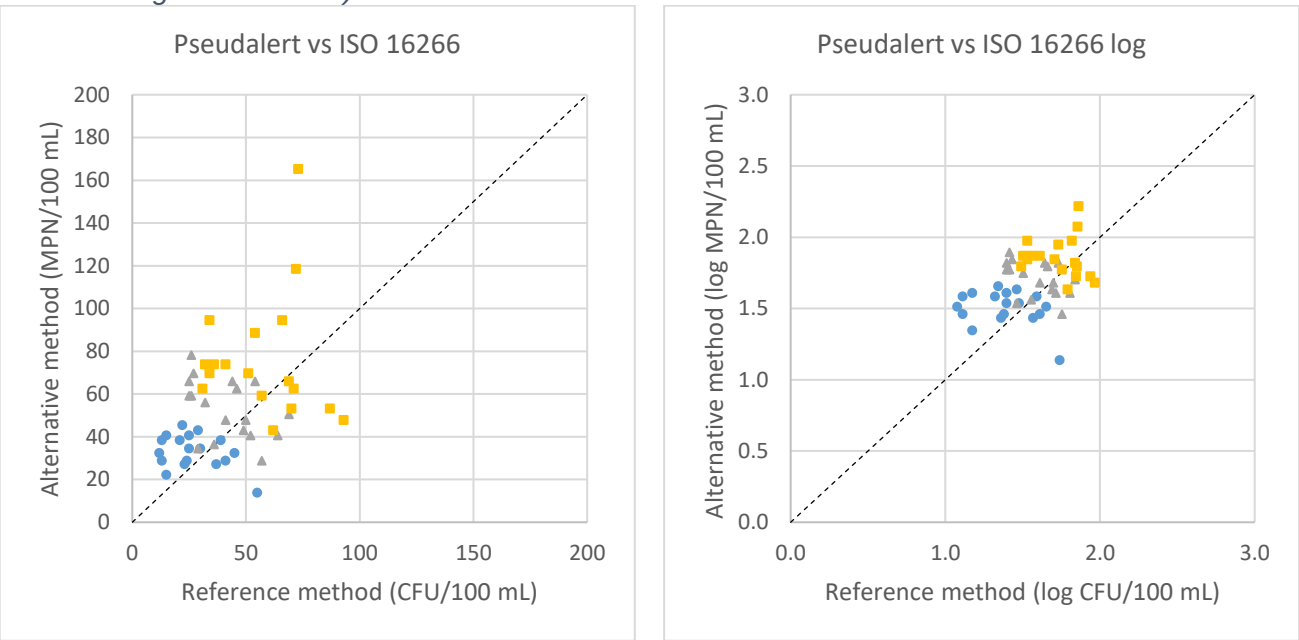
Table 14: results of the interlaboratory study (MPN and CFU / 100 mL)

Labora-tory	Level 1 (samples 4/7)				Level 2 (samples 5/6)				Level 3 (samples 1/3)			
	RM (CFU/100 mL)		AM (MPN/100 mL)		RM (CFU/100 mL)		AM (MPN/100 mL)		RM (CFU/100 mL)		AM (MPN/100 mL)	
	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2	R1	R2
A	38	32	<1	<1	46	26	1.0	<1	49	39	1.0	<1
B	38	34	<1	<1	32	47	<1	<1	80	62	<1	<1
C	28	25	6.4	200.5	44	30	5.3	200.5	46	47	7.5	200.5
D	17	9	4.2	1.0	32	29	2.0	3.1	27	45	5.3	2.0
E	22	12	45.3	32.4	26	26	78.2	59.1	34	36	94.5	73.8
F	25	21	40.6	38.4	46	54	62.4	65.9	54	66	88.5	94.5
G	15	23	40.6	27.1	27	25	69.7	65.9	72	31	118.4	62.4
H	25	24	34.4	28.8	41	44	47.8	65.9	62	34	42.9	69.7
I	38	28	<1	<1	54	58	<1	<1	79	84	<1	<1
J	41	55	28.8	13.7	57	52	28.8	40.6	70	87	53.1	53.1
K	15	29	22.2	42.9	29	36	34.4	36.4	51	57	69.7	59.1
L	13	13	38.4	28.8	25	32	59.1	56.0	32	41	73.8	73.8
M	30	39	34.4	38.4	50	49	47.8	42.9	71	73	62.4	165.2
N	37	45	27.1	32.4	64	69	40.6	50.4	93	69	47.8	65.9

Laboratories A and I incubated the Quanti-Tray at 37°C instead of 38±0.5°C as described in the Pseudalert method.
Laboratories B and D didn't count the wells with a weak fluorescence at 24 hours as described in the alternative method.
The laboratory C has aberrant results. For each level of contamination (L0, L1; L2 and L3), the alternative method presents one repetition inferior to 10 MPN / 100 mL while the other was superior to 200.5 MPN / 100 mL.

The Technical Board accepted to exclude the laboratories A, B, C, D and I of the statistical analysis of the data.
Despite of results a little high at level 1, the Technical Board accepted the data of the interlaboratory study.
The data obtained by the nine remaining laboratories are presented in the two dimensional graphs of the figure 3 in MPN and CFU /100 mL and in log MPN and log CFU / 100mL (y = x in dotted line).

Figure 3: two-dimensional graphs for the interlaboratory study (in MPN and CFU /100 mL and in log MPN and log CFU/100 mL)



3.2.2 Enumerations of culturable microorganisms

For the whole laboratories, the enumerations at 22°C vary between <1 and 7 CFU/mL. Concerning the enumerations at 36°C, the results were varying between <1 and 3 CFU/mL.
The results of each lab are shown in appendix 9.

3.2.3 Statistical interpretation

- **Bias**

Table 15 presents the target value, the mean, and the bias for each level of contamination.

Table 15: calculations of the bias of the alternative method

Values	MPN / 100 mL			log MPN/100 mL		
Levels	**1 - low**	**2 - medium**	**3 - high**	**1 - low**	**2 - medium**	**3 - high**
Target value	24.50	42.50	59.50	1.39	1.63	1.77
Mean	33.04	52.88	76.03	1.50	1.71	1.86
Relative bias	0.35	0.24	0.28	8.31%	4.92%	4.66%
Bias	1.35	1.24	1.28	0.12	0.08	0.08

The accuracy is estimated by the bias which varies between 1.24 MPN / 100 mL and 1.35 MPN / 100 mL and 0.08 log MPN / 100 mL and log 0.12 log MPN / 100 mL.
As a reminder, the bias obtained during the comparative study was 2.8 MPN / 100 mL and 0.047 log MPN / 100 mL.

- **Accuracy profile**

Tables 16 and 17 show the tolerance values and limits of the alternative method for the different values of probability of tolerance and the limits of acceptability, respectively in MPN / 100 mL and log MPN / 100 mL.

Table 16: tolerance values for the alternative method (MPN / 100 mL)

Tolerance probability	Parameters	UFC / 100 mL		
		Low	Medium	High
80%	Low tolerance value	21.96	32.61	36.05
	High tolerance value	44.12	73.15	116.01
	Low tolerance limit	**90%**	**77%**	**61%**
	High tolerance limit	**180%**	**172%**	**195%**

Table 17: tolerance values for the alternative method (log MPN / 100 mL)

Tolerance probability	Parameters	log MPN/100 mL		
		Low	Medium	High
90%	Low tolerance value	1.24	1.42	1.55
	High tolerance value	1.78	2.00	2.17
	Low tolerance limit	**- 0.15**	**- 0.20**	**-0.23**
	High tolerance limit	**0.38**	**0.37**	**0.39**

Figures 4 and 5 present the accuracy profiles.

Figure 4: accuracy profile (MPN / 100 mL) for a tolerance probability at 80% and acceptability limits at 100 %

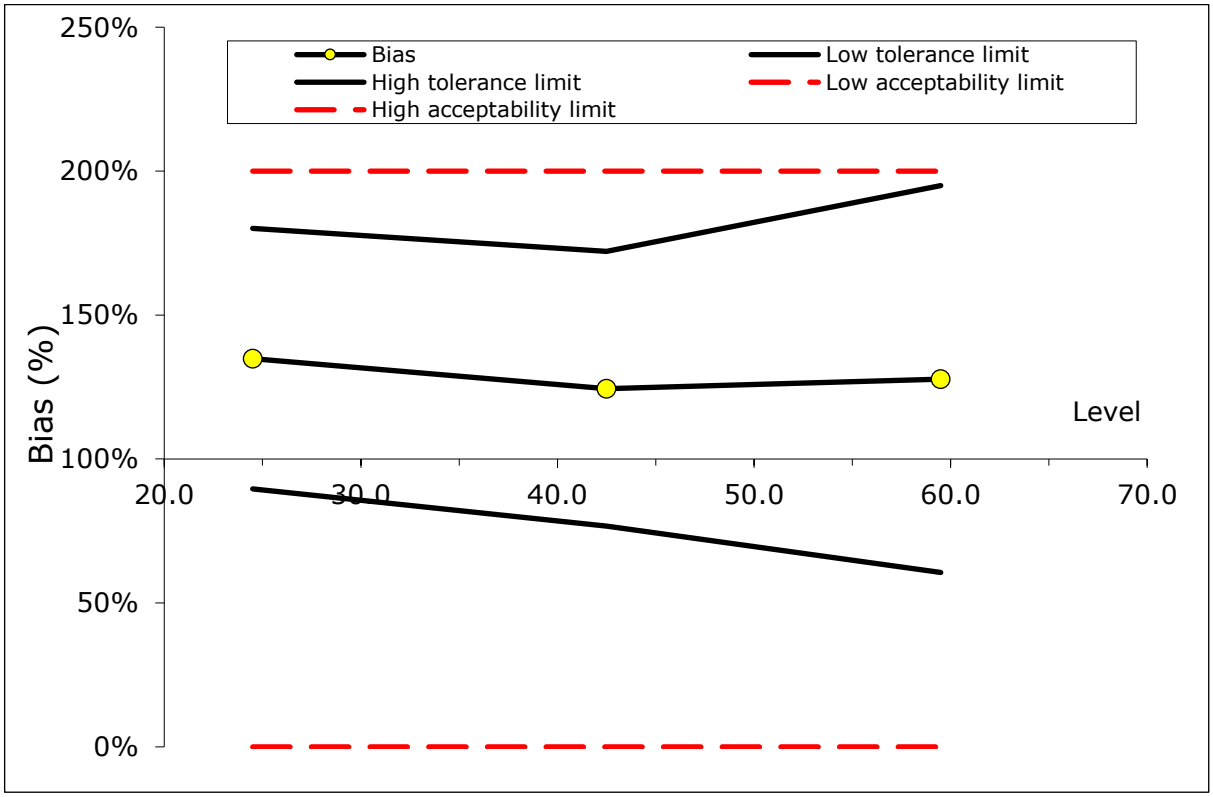
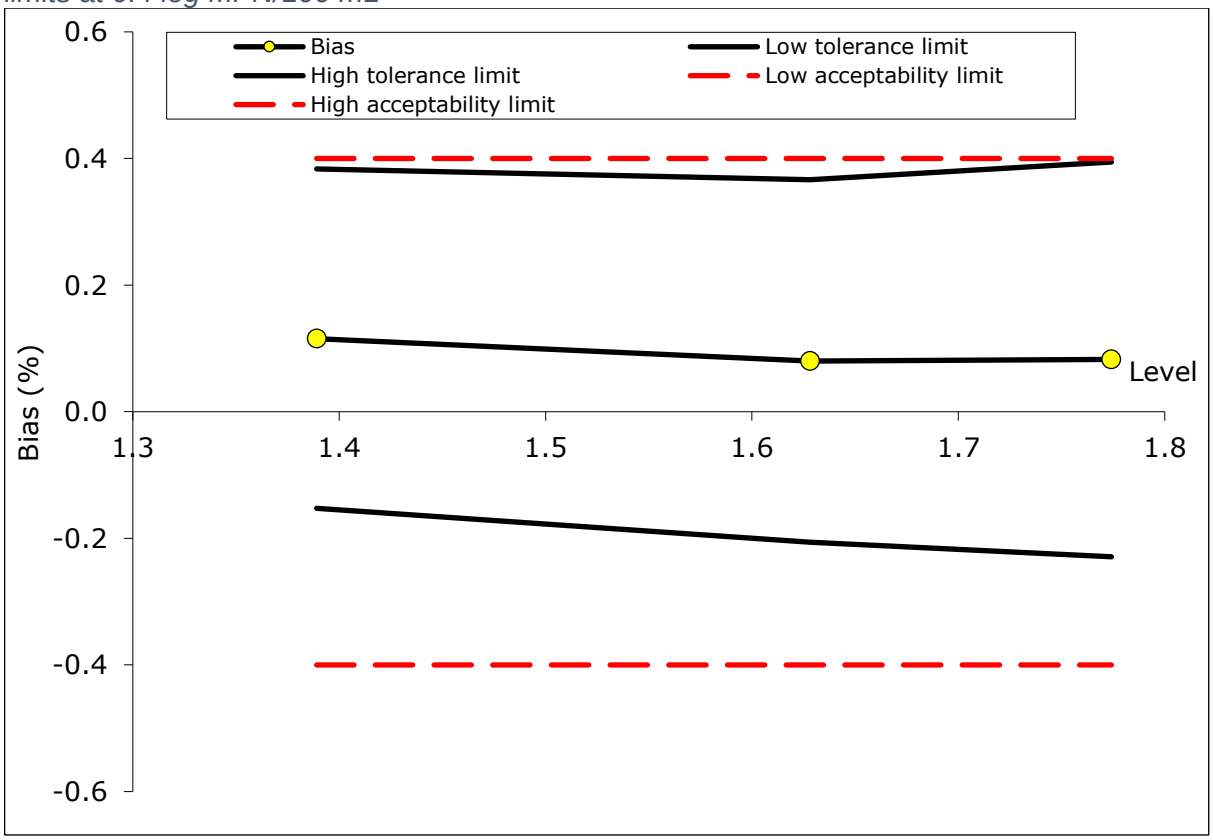


Figure 5: accuracy profile (log MPN / 100 mL) for a tolerance probability at 95% and acceptability limits at 0.4 log MPN/100 mL



For all the contamination levels, the tolerance interval is comprised between the acceptability interval for a 80% tolerance probability with acceptability limits at 100 % and between the acceptability interval for a 95% tolerance probability and a limit at 0,4 log MPN/100 mL.

3.2.4 Conclusion

The bias of the alternative method is relatively stable from the low level of contamination to the high level of contamination.

For all levels of contamination, the tolerance limits are between the limits of acceptability, meaning that at least 95% of the results will be between the limits of acceptability as defined at 0.4 log MPN/100 mL.

3.3 Conclusion

The performances of the **Pseudalert ®/ Quanti-tray®** method comply with the requirements of NF EN ISO 16266: 2008 and the AFNOR validation protocol: Validation protocol for an alternative commercial method as compared with a reference method (revision 2 – May 2013)

4 Bibliographical studies

4.1 Publications

Nine studies were published between 2010 and 2014.

Bédard E, Charron D, Lalancette C, Déziel E, Prévost M. **Jul 2014**. “Recovery of Pseudomonas aeruginosa culturability following copper- and chlorine-induced stress” *FEMS Microbiol Lett; 356 (2):226-34*

D. P. Sartory, M. Brewer, A. Beswick and D. Steggles **December 2015**. “Evaluation of the Pseudalert/Quanti-Tray MPN Test for the Rapid Enumeration of Pseudomonas aeruginosa in Swimming Pool and Spa Pool Waters” *Curr Microbiology 71:699-705*

David P. Sartory, Danièle Pauly, Nathalie Garrec, Lucia Bonadonna, Maurizio Semproni, Christiane Schell, Annika Reimann, Susan J. Firth, Christopher Thom, Philippe Hartemann, Martin Exner, Henning Baldauf, Susanne Lee and John V. Lee **13.2 2015.** “Evaluation of an MPN test for the rapid enumeration of Pseudomonas aeruginosa in hospital waters” *Journal of Water and Health*

Dominique Charron, MScA; Emilie Bédard, MScA; Cindy Lalancette, PhD; Céline Laferrière, MD; Michèle Prévost, PhD **March 2015.** “Impact of Electronic Faucets and Water Quality on the Occurrence of Pseudomonas aeruginosa in Water: A Multi-Hospital Study” *Infection Control & Hospital Epidemiology, Vol. 36, No. 3*

F. M. Schets, H. H. J. L. van den Berg, R. Baan, G. Lynch and A. M. de Roda Husman **12.4 2014.** “Pseudomonas aeruginosa on vinyl-canvas inflatables and foam teaching aids in swimming pools” *Journal of Water and Health*

Lawson R., Ruddle S., Calvert J. Oakland Calvert Consultants/Latis Scientific Ltd, Chair PWTAG, **April 9- 12 2013.** “The Evaluation of 24 Hour Pseudomonas aeruginosa Assay Techniques, using a Commercially Available Test Kit (Pseudalert)” United Kingdom - *Fifth International Conference Swimming Pool & Spa Abstract Book, Pg 20, Istituto Superiore di Sanità and Università di Roma Foro Italico Rome,; http://www.iss.it/binary/publ/cont/13_C1.pdf*

M. Semproni, R. Briancesco, G. Gianfranceschi, S. Giampaoli, R. Paradiso, V. Romano Spica, F. Valerianie L. Bonadonna **Jan- Feb 2014 .** “Comparison of cultural methods for the recovery of Pseudomonas aeruginosa: the UNI EN ISO 16266 reference method and the alternative method Pseudalert®” *Ann Ig; 26(1):110-8*

Ngwa, G.A., Schop, R., Chow, J., Lukic, L., McKague, K., **February 2017** “Comparative detection and recovery of Pseudomonas aeruginosa by membrane filtration and a Most Probable Number technique.” *Journal of Microbiological Methods; 133:76-81*

Steffen Schneider, Hessenwasser GmbH & Co. KG **July 2011.** “Bestimmug von Pseudomonas aeruginosa in Schwimm-und Badebeckenwasser mit dem Pseudalert-Verfahren Besondere Eignung zur Unterschung von Naturfreibadern?” Der Hygieneinspektor, Auszug der Seiten 44-47; Beitrag

IDEXX **October 2010**.“Comparison of the performance of the IDEXX Pseudalert test against SM 9213E at recovering confirmed Pseudomonas aeruginosa from pool/spa water samples”; *technical note (summary)*

IDEXX **2010.** “Comparison of the performance of the IDEXX Pseudalert test against the EN ISO 162266:2008 method recovering confirmed Pseudomonas aeruginosa from pool/spa water samples”; *technical note (summary)*

IDEXX **November 2010.** “Comparison of the performance of IDEXX Pseudalert test against the Millipore and Centrimide membrane filtration methods at recovering confirmed Pseudomonas aeruginosa from bottled water samples”; *technical note (summary)*

IDEXX **February 2011**. “Comparing the performance of the IDEXX Pseudalert test against a modified version of the ISO 16266:2006 method used at a major bottled water company for the recovery and specific detection of confirmed Pseudomonas aeruginosa from water samples.”; *technical note (summary)*

IDEXX **2010**. “Performance comparison of the IDEXX Pseudalert method versus the Whatman Pseudomonas Broth method at recovering confirmed Pseudomonas aeruginosa from bottled water samples”; *technical note (summary)*

Maynard, Elise, **2015**. “The Use of Pseudalert for the Routine Analysis of Water Samples by Engineers” *Healthcare Infection Society 3393*

In these articles, the performances of **Pseudalert ®/ Quanti-tray®** method are satisfactory. They are compared to the reference method ISO 16266 (2006 and 2008) or SM 9213E, Whatman Pseudomonas broth method, MoDW part 8 (Uk the Microbiology of Drinking Water), Milipore™ and Cetrimide membrane filtration methods

4.2 External validations and recognitions

The external recognitions of **Pseudalert ®/ Quanti-tray®** method are summarized in the table below:

Approval	Body	Nature of validation protocol
May 2015	Umwelt Bundesamt (UBA) (Germany)	- Approval of Pseudalert/Quanti-Tray for compliance testing of drinking water, pools and spas - Evaluation of the results of comparative tests according to DIN EN ISO 17994 versus the DIN EN ISO 16266 method
December 2016	The National Public Health and Medical Service Office (Hungry)	- Approval of Pseudalert/Quanti-Tray for Compliance Testing of Drinking Water - Evaluation of the results of comparative tests according to EN ISO 17994 versus the EN ISO 16266 method done by National Public Health Center: Laboratory of Water Hygiene
June 2014	National Institute of Health (Italy)	- Approval of Pseudalert for Compliance Testing of Pool and Water Samples - Approval based on several equivalency studies performed in Italy and Europe, done according to the criteria of ISO 17994
May 2012	Department of health and senior services Division of epidemiology, environmental and occupational health	- Approval of Pseudalert for P. aeruginosa in Spa/Hot Tub - Approval based on an external study where the laboratory compared Pseudalert to Standard Methods 9213E

Pseudalert ®/ Quanti-tray® method has been published as the International Organization for Standardization (ISO): ISO 16266-2:2018; Water Quality – Detection and enumeration of Pseudomonas aeruginosa – Part 2: Most probable number method

Due to its performance, **Pseudalert ®/ Quanti-tray®** method is also recommended as standard methods in:

- UK Standing Committee of Analysts (SCA) Blue Books: The Microbiology of Recreational and Environmental Waters (2015) – Part7 - Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa
- UK Standing Committee of Analysts (SCA) Blue Books: The Microbiology of Drinking Water (2015) – Part 8 - Methods for the isolation and enumeration of Aeromonas and Pseudomonas aeruginosa.

Done at Thury-Harcourt, February 07, 2024
Mickaël MORVAN
Research & Development Engineer

Appendix 1: alternative method protocol

Protocol for a test portion of 100 mL

Step 1

Add contents of one snap pack to 100 mL of sample
Cap vessel and shake until dissolved
Pour sample/reagent mixture into a Quanti-Tray®

Step 2

Seal in a Quanti-Tray® Sealer

Step 3

Place the sealed tray in a 38±0.5°C incubator for 24 to 28 hours

Step 4

Count the number of fluorescence wells with UV lamp at 365 nm

Step 5

Results:
Refer to the MPN table provided with the trays to obtain a Most Probable Number

Expressed results in MPN Pseudomonas aeruginosa / 100 mL of sample

Protocol for a test portion of 250 mL

Step 1

Fill 3 vessels with the sample:
- the first at 100 mL,
- the second at 100 mL,
- the third at 50 mL and add 50 mL of sterile water

Add contents of one snap pack to each vessel
Cap vessels and shake until dissolved
Pour sample/reagent mixture into a Quanti-Tray® for each vessel

Step 2

Seal in a Quanti-Tray® Sealer

Step 3

Place the sealed trays in a 38±0.5°C incubator for 24 to 28 hours

Step 4

Count the number of fluorescence wells with UV lamp at 365 nm per Quanti-Tray®
Add the number of positive wells on the 3 trays

Step 5

Results :
Refer to the MPN table provided with the trays to obtain a Most Probable Number

Expressed results in MPN Pseudomonas aeruginosa / 250 mL of sample

Appendix 2: reference method protocol

NF EN ISO 16266:2008

Detection and enumeration of Pseudomonas aeruginosa
- Method by membrane filtration

Membrane filtration

Filter 100 or 250 mL of sample through membrane filter
Place membrane on a Petri dish containing CN agar

Incubation

Incubate at (36±2)°C for (44±4)h

Reading

Examine the membranes after (22±2)h and (44±4)h of incubation

Case n°1:
Count colonies with a blue/green colour (pyocyanine (+))
as confirmed *Pseudomonas aeruginosa*

Case n°2:
Count colonies pyocyanine (-) and fluorescence (+)
as presumptive *Pseudomonas aeruginosa*
Confirmation : Inoculate an acetamide broth
Count as confirmed *Pseudomonas aeruginosa*, colonies producing ammonia

Case n°3:
Count all other reddish brown pigmented colonies and fluorescence (-)
Confirmation: oxidase, acetamide broth and King's B media
Count as confirmed *Pseudomonas aeruginosa*, colonies oxidase (+), ammonia (+) and fluorescence on King's B media

Results: CFU/ 100 or 250 mL

Appendix 3: bacterial stress

Code	Strain	Origin	Stress applied	Stress intensity	Samples spiked
PSE 1.14	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	>1,4	19/22/24
PSE 1.21	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	>1,7	20/23/25
PSE 1.29	*Pseudomonas aeruginosa*	ATCC 27853	hypochloride treatement 20 min + LT100 broth	>1,0	21
PSE 1.1 —	*Pseudomonas aeruginosa*	Eau	6 days at -80°C + 20 min 56°C	1,9	26/30/34/55
PSE 1.4	*Pseudomonas aeruginosa*	Eau de fontaine	6 days at -80°C + 20 min 56°C	1,7	27/31/35
PSE 1.5	*Pseudomonas aeruginosa*	Eau de fontaine	6 days at -80°C + 20 min 56°C	2,6	28/32/36
PSE 1.6	*Pseudomonas aeruginosa*	Eau de fontaine	3 cycles freezing/defrosting	3,7	29/33/37/51
PSE 1.7	*Pseudomonas aeruginosa*	Eau d'effluent	3 cycles freezing/defrosting	>3,9	38
PSE 1.8	*Pseudomonas aeruginosa*	Eau de Fontaine	3 cycles freezing/defrosting	1,5	39/40/42/52
PSE 1.9	*Pseudomonas aeruginosa*	Eau	3 cycles freezing/defrosting	2,5	41/43/45/53
PSE 1.10	*Pseudomonas aeruginosa*	Eau	3 cycles freezing/defrosting	1,2	44/46/47/48
PSE 1.25	*Pseudomonas aeruginosa*	Eau	15 min at -80°C + 20 min at 569C	1,1	15/17
PSE 1.16	*Pseudomonas aeruginosa*	Eau	15 min at -80°C + 20 min at 569C	2,9	16/49/50
PSE 1.27	*Pseudomonas aeruginosa*	Eau	20 min at -80°C + 20 min at 56°C	0,7	54
PSE 1.13	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	1,4	1/4
PSE 1.20	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	1,7	2/5
———	———————	———	———————————	——	≠
PSE 1.22	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	>1,8	6/14
PSE 1.23	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	>2,5	7/11
PSE 1.26	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	0,7	8/12
PSE 1.15	*Pseudomonas aeruginosa*	Eau	10 min at -80°C + 10 min at - 56°C	1,8	9/13
PSE 1.6	*Pseudomonas aeruginosa*	Eau de fontaine	hypochloride treatement 20 min + LT100 broth	1,3	62/63/72/73
PSE 1.19	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	0,4	70/71
PSE 1.24	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 10 min + LT100 broth	1,1	58/59/64/65/74/75
PSE 1.28	*Pseudomonas aeruginosa*	ATCC 10145	hypochloride treatement 10 min + LT100 broth	0,7	60/61/66/67
PSE 1.5	*Pseudomonas aeruginosa*	Eau de fontaine	hypochloride treatement 10 min + LT100 broth	0,9	76/77/84/85
PSE 1.7	*Pseudomonas aeruginosa*	Eau d'effluent	hypochloride treatement 10 min + LT100 broth	1,4	78/79/86/87
PSE 1.14	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	0,7	80/81/88/89
PSE 1.16	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	0,9	82/83/90/91
PSE 1.17	*Pseudomonas aeruginosa*	Eau	hypochloride treatement 20 min + LT100 broth	1,1	96/97
PSE 1.18	*Pseudomonas aeruginosa*	Eau	5 min at 56°C + 30 min at - 80°C	>5,4	92/93
PSE 1.23	*Pseudomonas aeruginosa*	Eau	5 min at 56°C + 30 min at - 80°C	>3,1	94/95
PSE 1.12	*Pseudomonas aeruginosa*	Eau de lac	hypochloride treatement 5 min + LT100 broth	1,4	68/69
PSE 1.12	*Pseudomonas aeruginosa*	Eau de lac	hypochloride treatement 10 min + LT100 broth	>5,8	56/57

Code	Strain	Origin	Stress applied	Stress intensity	Samples spiked
202009-2424	*Pseudomonas aeruginosa*	WDCM 00025	hypochloride treatment 20 min + LT100 broth	1,2	139/140/141/142/149
20202009-2477	*Pseudomonas aeruginosa*	Eau de fontaine	hypochloride treatment 10 min + LT100 broth	0,8	143/144/145/150
202010-241	*Pseudomonas aeruginosa*	Eau	hypochloride treatment 10 min + LT100 broth	0,7	146/147/148

Appendix 4: relative accuracy results

Renewal 2024 - Taking into account new ISO 8199:2018 requirements

Raw results for human drinking water category

Total raw results for human drinking waters category

Number	Sample	Strain	Level (UFC/100mL or UFC/250mL)	Alternative method						Reference method (*)									
				Replicate 1			Replicate 2			Replicate 1					Replicate 2				
				Nb of fluo wells	MPN/100mL or MPN/250mL	LOG (NPP/100 mL) or Log (NPP/250 mL)	Nb of fluo wells	MPN/100mL or MPN/250mL	LOG (NPP/100 mL) or Log (NPP/250 mL)	Blue green colonies	Nb of fluorescent colonies (non blue-green)	% of ammonium +	MPN/100mL or MPN/250mL	LOG (NPP/100 mL) or Log (NPP/250 mL)	Blue green colonies	Nb of fluorescent colonies (non blue-green)	% of ammonium +	MPN/100mL or MPN/250mL	LOG (NPP/100 mL) or Log (NPP/250 mL)
4	Tap water 1	PSE 1.13	42	30	45,3	1,656	32	50,4	1,702	24			24	1,380	34			34	1,531
5	Packaged water 1	PSE 1.20	19	24	32,4	1,511	19	23,8	1,377	54			54	1,732	47			47	1,672
6	Packaged water 2	PSE 1.22	41	10/12/5	30,1	1,479	1/8/8	18,4	1,265	>150			>150	>2,176	>150			>150	>2,176
7	Packaged water 3	PSE 1.26	7	4/1/0	5,2	0,716	3/1/1	5,1	0,708		103	100%	103	2,013		80	100%	80	1,903
8	Packaged water 4	PSE 1.26	36	31/15/8	74,3	1,871	20/23/7	63,5	1,803	97			97	1,987	72			72	1,857
9	Packaged water 5	PSE 1.15	75	13/17/9	45,6	1,659	17/12/5	39,7	1,599		72	100%	72	1,857		90	100%	90	1,954
11	Tap water 2	PSE 1.23	48	10	11,1	1,045	7	7,5	0,875	2			2	0,301	4			4	0,602
12	Tap water 3	PSE 1.26	42	5	5,3	0,724	3	3,1	0,491	<1			<1	0,000	<1			<1	0,000
13	Tap water 4	PSE 1.15	79	1	1,0	0,000	4	4,2	0,623	<1			<1	0,000	<1			<1	0,000
14	Tap water 5	PSE 1.22	41	9	9,9	0,996	8	8,7	0,940	6			6	0,778	8			8	0,903
15	Packaged water 6	PSE 1.25	77	13/7/3	25,6	1,408	8/15/4	30,7	1,487	20			20	1,301	26			26	1,415
16	Packaged water 7	PSE 1.16	19	4/2/1	7,2	0,857	4/2/3	9,3	0,968	17			17	1,230	13			13	1,114
17	Tap water 6	PSE 1.25	77	21	27,1	1,433	22	28,8	1,459	47			47	1,672	52			52	1,716
19	Packaged water 8	PSE 1.14	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
20	Packaged water 9	PSE 1.21	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
21	Packaged water 10	PSE 1.29	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
22	Packaged water 11	PSE 1.14	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
23	Packaged water 12	PSE 1.21	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
24	Packaged water 13	PSE 1.14	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
25	Packaged water 14	PSE 1.21	0	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
26	Packaged water 15	PSE 1.1	42	0/0/0	<1	0,000	0/0/0	<1	0,000		8	100%	8	0,000		5	100%	5	0,000
27	Packaged water 16	PSE 1.4	29	5/10/3	19,5	1,290	3/6/7	17,0	1,230	8			8	0,903	8			8	0,903
28	Packaged water 17	PSE 1.5	22	1/4/0	5,2	0,716	5/4/2	20,5	1,312		1	100%	1	0,000		2	100%	2	0,301
29	Tap water 7	PSE.1.6	20	10	11,1	1,045	12	13,7	1,137		4	100%	4	0,602		10	80%	8	0,903
30	Tap water 8	PSE 1.1	42	0	<1	0,000	0	<1	0,000	<1			<1	0,000	<1			<1	0,000
31	Tap water 9	PSE 1.4	29	16	19,2	1,283	17	20,7	1,316	15			15	1,176	12			12	1,079
32	Tap water 10	PSE 1.5	22	0	<1	0,000	0	<1	0,000		1	100%	1	0,000		<1		<1	0,000
33	Tap water 11	PSE 1.6	20	7	7,5	0,875	12	13,7	1,137		11	100%	11	1,041		5	100%	5	0,699
34	Tap water 12	PSE 1.1	42	5	5,3	0,724	1	1,0	0,000		4	100%	4	0,602		4	100%	4	0,602
35	Borehole water 1	PSE 1.4	29	17	20,7	1,316	18	22,2	1,346	14			14	1,146	14			14	1,146
36	Borehole water 2	PSE 1.5	22	3	3,1	0,491	5	5,3	0,724		1	100%	1	0,000		1	100%	1	0,000
37	Borehole water 3	PSE 1.6	20	9	9,9	0,996	10	11,1	1,045		6	100%	6	0,778		1	100%	1	0,000
38	Borehole water 4	PSE 1.7	>300	0/0/0	<1	0,000	0/0/0	<1	0,000		11	80%	8,8	0,944		10	100%	10	1,000
39	Tap water 13	PSE 1.8	34	21	27,1	1,433	23	30,6	1,486	20			20	1,301	22	-		22	1,342
40	Packaged water 18	PSE 1.8	63	26/22/9	75,1	1,876	20/23/10	67,1	1,827	59			59	1,771	56			56	1,748
41	Packaged water 19	PSE 1.9	19	9/6/4	20,5	1,312	6/5/2	13,7	1,137		3	100%	3	0,477		3	100%	1	0,000
42	Packaged water 20	PSE 1.8	63	9/17/18	34,8	1,542	21/21/13	69,2	1,840	66			66	1,820	52			52	1,716
43	Packaged water 21	PSE 1.9	19	5/7/5	18,1	1,258	6/7/4	18,1	1,258		15	100%	15	1,176		8	100%	8	0,903
44	Packaged water 22	PSE 1.10	42	0/0/0	<1	0,000	0/0/0	<1	0,000		35	100%	35	1,544		24	100%	24	1,380
45	Packaged water 23	PSE 1.9	19	0/0/0	<1	0,000	0/0/0	<1	0,000		21	100%	21	1,322		9	100%	9	0,954
46	~~Spring~~ Fountain water 1	PSE 1.10	70	10	11,1	1,045	14	16,4	1,215		28	100%	28	1,447		30	100%	30	1,477
47	~~Spring~~ Fountain water 2	PSE 1.10	70	5	5,3	0,724	9	9,9	0,996		33	100%	33	1,519		38	100%	38	1,580
48	~~Spring~~ Fountain water 3	PSE 1.10	70	9	9,9	0,996	10	11,1	1,045		43	100%	43	1,633		49	100%	49	1,690
49	Packaged water 24	PSE 1.16	154	7/13/15	27,8	1,444	9/12/1	24,6	1,391	46			46	1,663	49			49	1,690
50	Packaged water 25	PSE 1.16	154	21/23/8	66,4	1,822	17/17/5	46,7	1,669	86			86	1,934	93			93	1,968
51	Packaged water 26	PSE 1.6	98	37/39/30	185,0	2,267	11/26/24	81,2	1,910		54	100%	54	1,732		62	100%	62	1,792
52	Packaged water 27	PSE 1.8	81	21/28/17	172,4	2,237	41/35/34	198,2	2,297	140			140	2,146	144			144	2,158
53	Packaged water 28	PSE 1.9	99	21/28//17	88,4	1,946	38/33/33	175,9	2,245	124			124	2,093	106			106	2,025
54	Tap water 14	PSE 1.27	113	18	22,2	1,346	24	32,4	1,511	52			52	1,716	54			54	1,732
55	~~Spring~~ Fountain water 4	PSE 1.1	92	8	8,7	0,940	4	4,2	0,623	55			55	1,740	51			51	1,708
92	Packaged water 29	PSE 1.17	48	38/33/25	157,2	2,196	34/34/22	140,8	2,149		100	100%	100	2,000		72	100%	72	1,857
93	Packaged water 30	PSE 1.17	68	8/5/34	60,0	1,778	28/3/0	43,7	1,640		>150		>150	>2,176		145	100%	145	2,161
94	Packaged water 31	PSE 1.18	166	11/7/1	20,9	1,320	9/3/0	13,0	1,114		23	100%	23	1,362		35	100%	35	1,544
95	Packaged water 32	PSE 1.18	6	0/0/0	<1	0,000	0/0/0	<1	0,000		9	100%	9	0,954		2	100%	2	0,301
96	Packaged water 33	PSE 1.23	10	5/2/2	9,3	0,968	6/4/2	16,6	1,220		5	100%	5	0,699		15	100%	15	1,176
97	Packaged water 34	PSE 1.23	13	1/10/8	20,8	1,318	3/3/4	10,4	1,017		18	100%	18	1,255		14	100%	14	1,146

Total exploitable results for human drinking water category

Number	Sample	Strain	Level (CFU/100mL)	Alternative method						Reference method									
				Replicate 1			Replicate 2			Replicate 1					Replicate 2				
				Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)
4	Tap water 1	PSE 1.13	42	30	45.3	1.656	32	50.4	1.702	24			24	1.380	34			34	1.531
5	Packaged water 1	PSE 1.20	19	24	32.4	1.511	19	23.8	1.377	54			54	1.732	47			47	1.672
14	Tap water 5	PSE 1.22	41	9	9.9	0.996	8	8.7	0.940	6			6	0.778	8			8	0.903
15	Packaged water 6	PSE 1.25	77	13/7/3	25.6	1.408	8/15/4	30.7	1.487	20			20	1.301	26			26	1.415
16	Packaged water 7	PSE 1.16	19	4/2/1	7.2	0.857	4/2/3	9.3	0.968	17			17	1.230	13			13	1.114
17	Tap water 6	PSE 1.25	77	21	27.1	1.433	22	28.8	1.459	47			47	1.672	52			52	1.716
27	Packaged water 16	PSE 1.4	29	5/10/3	19.5	1.290	3/6/7	17	1.230	8			8	0.903	8			8	0.903
29	Tap water 7	PSE 1.6	20	10	11.1	1.045	12	13.7	1.137		4	100%	4	0.602		10	100%	10	1.000
31	Tap water 9	PSE 1.4	29	16	19.2	1.283	17	20.7	1.316	15			15	1.176	12			12	1.079
33	Tap water 11	PSE 1.6	20	7	7.5	0.875	12	13.7	1.137		11	100%	11	1.041		5	100%	5	0.699
34	Tap water 12	PSE 1.1	42	5	5.3	0.724	1	1	0.000		4	100%	4	0.602		4	100%	4	0.602
35	Borehole water 1	PSE 1.4	29	17	20.7	1.316	18	22.2	1.346	14			14	1.146	14			14	1.146
39	Tap water 15	PSE 1.8	34	21	27.1	1.433	23	30.6	1.486	20			20	1.301	22	-		22	1.342
40	Packaged water 18	PSE 1.8	63	26/22/9	75.1	1.876	20/23/10	67.1	1.827	59			59	1.771	56			56	1.748
42	Packaged water 20	PSE 1.8	63	9/17/18	34.8	1.542	21/21/13	69.2	1.840	66			66	1.820	52			52	1.716
43	Packaged water 21	PSE 1.9	19	5/7/5	18.1	1.258	6/7/4	18.1	1.258		15	100%	15	1.176		8	100%	8	0.903
46	Fountain water 1	PSE 1.10	70	10	11.1	1.045	14	16.4	1.215		28	100%	28	1.447		30	100%	30	1.477
47	Fountain water 2	PSE 1.10	70	5	5.3	0.724	9	9.9	0.996		33	100%	33	1.519		38	100%	38	1.580
48	Fountain water 3	PSE 1.10	70	9	9.9	0.996	10	11.1	1.045		43	100%	43	1.633		49	100%	49	1.690
49	Packaged water 24	PSE 1.16	154	7/13/15	27.8	1.444	9/12/1	24.6	1.391	46			46	1.663	49			49	1.690
51	Packaged water 26	PSE 1.6	98	37/39/30	185	2.267	11/26/24	81.2	1.910		54	100%	54	1.732		62	100%	62	1.792
54	Tap water 14	PSE 1.27	113	18	22.2	1.346	24	32.4	1.511	52			52	1.716	54			54	1.732
55	Fountain water 4	PSE 1.1	92	8	8.7	0.940	4	4.2	0.623	55			55	1.740	51			51	1.708
94	Packaged water 31	PSE 1.18	166	11/7/1	20.9	1.320	9/3/0	13	1.114		23	100%	23	1.362		35	100%	35	1.544
96	Packaged water 33	PSE 1.23	10	5/2/2	9.3	0.968	6/4/2	16.6	1.220		5	100%	5	0.699		15	100%	15	1.176
97	Packaged water 34	PSE 1.23	13	1/10/8	20.8	1.318	3/3/4	10.4	1.017		18	100%	18	1.255		14	100%	14	1.146

Relative accuracy – Human drinking category – CFU/ 100 or 250 mL

Méthode de référence				
Echantillon	Répétition 1	Répétition 2	M	SD
1	24	34	29.0	7.1
2	54	47	50.5	4.9
3	6	8	7.0	1.4
4	20	26	23.0	4.2
5	17	13	15.0	2.8
6	47	52	49.5	3.5
7	8	8	8.0	0.0
8	4	10	7.0	4.2
9	15	12	13.5	2.1
10	11	5	8.0	4.2
11	4	4	4.0	0.0
12	14	14	14.0	0.0
13	20	22	21.0	1.4
14	59	56	57.5	2.1
15	66	52	59.0	9.9
16	15	8	11.5	4.9
17	28	30	29.0	1.4
18	33	38	35.5	3.5
19	43	49	46.0	4.2
20	46	49	47.5	2.1
21	54	62	58.0	5.7
22	52	54	53.0	1.4
23	55	51	53.0	2.8
24	23	35	29.0	8.5
25	5	15	10.0	7.1
26	18	14	16.0	2.8

Méthode alternative				
Echantillon	Répétition 1	Répétition 2	M	SD
1	45.3	50.4	47.9	3.6
2	32.4	23.8	28.1	6.1
3	9.9	8.7	9.3	0.8
4	25.6	30.7	28.2	3.6
5	7.2	9.3	8.3	1.5
6	27.1	28.8	28.0	1.2
7	19.5	17.0	18.3	1.8
8	11.1	13.7	12.4	1.8
9	19.2	20.7	20.0	1.1
10	7.5	13.7	10.6	4.4
11	5.3	1.0	3.2	3.0
12	20.7	22.2	21.5	1.1
13	27.1	30.6	28.9	2.5
14	75.1	67.1	71.1	5.7
15	34.8	69.2	52.0	24.3
16	18.1	18.1	18.1	0.0
17	11.1	16.4	13.8	3.7
18	5.3	9.9	7.6	3.3
19	9.9	11.1	10.5	0.8
20	27.8	24.6	26.2	2.3
21	185.0	81.2	133.1	73.4
22	22.2	32.4	27.3	7.2
23	8.7	4.2	6.5	3.2
24	20.9	13.0	17.0	5.6
25	9.3	16.6	13.0	5.2
26	21	10	15.6	7.4

Différence
18.9
-22.4
2.3
5.2
-6.8
-21.6
10.3
5.4
6.5
2.6
-0.9
7.5
7.9
13.6
-7.0
6.6
-15.3
-27.9
-35.5
-21.3
75.1
-25.7
-46.6
-12.1
3.0
-0.4

q= 26
n= 2
N=qn= 52

Mx= 29.540
MEDx= 29.000
SDbx= 19.534

MEDwx= 3.536
SDwx= 2.605
rob.SDwx= 5.242

My= 25.994
MEDy= 18.175
SDby= 26.736

MEDwy= 3.217
SDwy= 14.361
rob.SDwy= 4.770

M= -3.025
MED= 0.950

Choix de la méthode

GMFR

R= 5.512
rob.R= 0.910

Sx= 19.588
Sy= 29.203

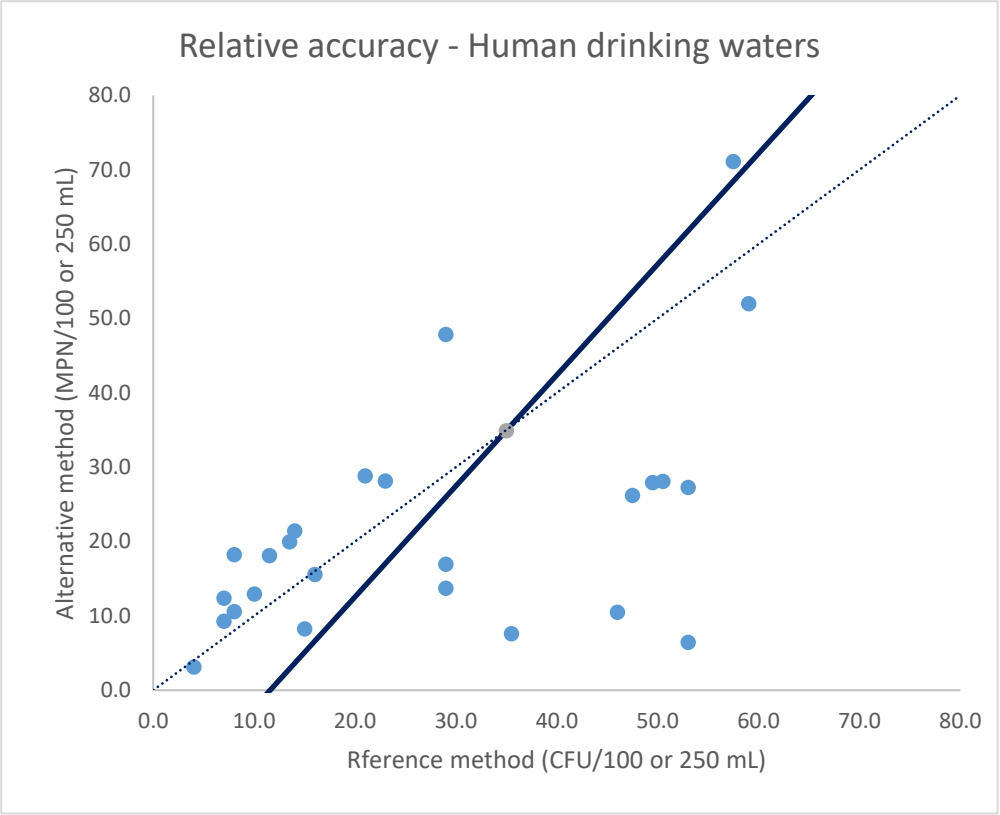
r= 0.494
b= 1.491
a= -17.269

Res. SEM= 132.235
Res.SD= 187.009

S(b)= 1.350 **t(b)=** 0.364 -2.060**<0.364**<2.060 **Hypothèse b=1 validée**
S(a)= 48.266 **t(a)=** 0.358 -2.060**<0.358**<2.060 **Hypothèse a=0 validée**

Est. y	Dév.
26.0	21.9
58.0	-29.9
-6.8	16.1
17.0	11.1
5.1	3.2
56.5	-28.6
-5.3	23.6
-6.8	19.2
2.9	17.1
-5.3	15.9
-11.3	14.5
3.6	17.8
14.0	14.8
68.5	2.6
70.7	-18.7
-0.1	18.2
26.0	-12.2
35.7	-28.1
51.3	-40.8
53.5	-27.3
69.2	63.9
61.7	-34.4
61.7	-55.3
26.0	-9.0
-2.4	15.3
6.6	9.0

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Relative accuracy – Human drinking category – log CFU/ 100 or 250 mL

Méthode de référence				
Echantillon	Répétition 1	Répétition 2	M	SD
1	1.380	1.531	1.456	0.107
2	1.732	1.672	1.702	0.043
3	0.778	0.903	0.841	0.088
4	1.301	1.415	1.358	0.081
5	1.230	1.114	1.172	0.082
6	1.672	1.716	1.694	0.031
7	0.903	0.903	0.903	0.000
8	0.602	1.000	0.801	0.281
9	1.176	1.079	1.128	0.069
10	1.041	0.699	0.870	0.242
11	0.602	0.602	0.602	0.000
12	1.146	1.146	1.146	0.000
13	1.301	1.342	1.322	0.029
14	1.771	1.748	1.760	0.016
15	1.820	1.716	1.768	0.073
16	1.176	0.903	1.040	0.193
17	1.447	1.477	1.462	0.021
18	1.519	1.580	1.549	0.043
19	1.633	1.690	1.662	0.040
20	1.663	1.690	1.676	0.019
21	1.732	1.792	1.762	0.042
22	1.716	1.732	1.724	0.012
23	1.740	1.708	1.724	0.023
24	1.362	1.544	1.453	0.129
25	0.699	1.176	0.938	0.337
26	1.255	1.146	1.201	0.077

Méthode alternative				
Echantillon	Répétition 1	Répétition 2	M	SD
1	1.656	1.702	1.679	0.033
2	1.511	1.377	1.444	0.095
3	0.996	0.940	0.968	0.040
4	1.408	1.487	1.448	0.056
5	0.857	0.968	0.913	0.079
6	1.433	1.459	1.446	0.019
7	1.290	1.230	1.260	0.042
8	1.045	1.137	1.091	0.065
9	1.283	1.316	1.300	0.023
10	0.875	1.137	1.006	0.185
11	0.724	0.000	0.362	0.512
12	1.316	1.346	1.331	0.021
13	1.433	1.486	1.459	0.037
14	1.876	1.827	1.851	0.035
15	1.542	1.840	1.691	0.211
16	1.258	1.258	1.258	0.000
17	1.045	1.215	1.130	0.120
18	0.724	0.996	0.860	0.192
19	0.996	1.045	1.020	0.035
20	1.444	1.391	1.417	0.038
21	2.267	1.910	2.088	0.253
22	1.346	1.511	1.428	0.116
23	0.940	0.623	0.781	0.224
24	1.320	1.114	1.217	0.146
25	0.968	1.220	1.094	0.178
26	1.318	1.017	1.168	0.213

Différence
0.223
-0.259
0.127
0.090
-0.259
-0.248
0.357
0.290
0.172
0.136
-0.240
0.185
0.138
0.092
-0.077
0.218
-0.332
-0.689
-0.641
-0.259
0.326
-0.296
-0.943
-0.236
0.157
-0.033

q= 26
n= 2
N=qn= 52

Mx= 1.335
MEDx= 1.405
SDbx= 0.362

MEDwx= 0.043
SDwx= 0.089
rob.SDwx= 0.064

My= 1.258
MEDy= 1.259
SDby= 0.358

MEDwy= 0.072
SDwy= 0.112
rob.SDwy= 0.106

M= -0.077
MED= 0.028

Choix de la méthode

GMFR

R= 1.257
rob.R= 1.666

Sx= 0.368
Sy= 0.372

r= 0.541
b= 1.012
a= -0.093

Res. SEM= 1.675
Res.SD= 2.369

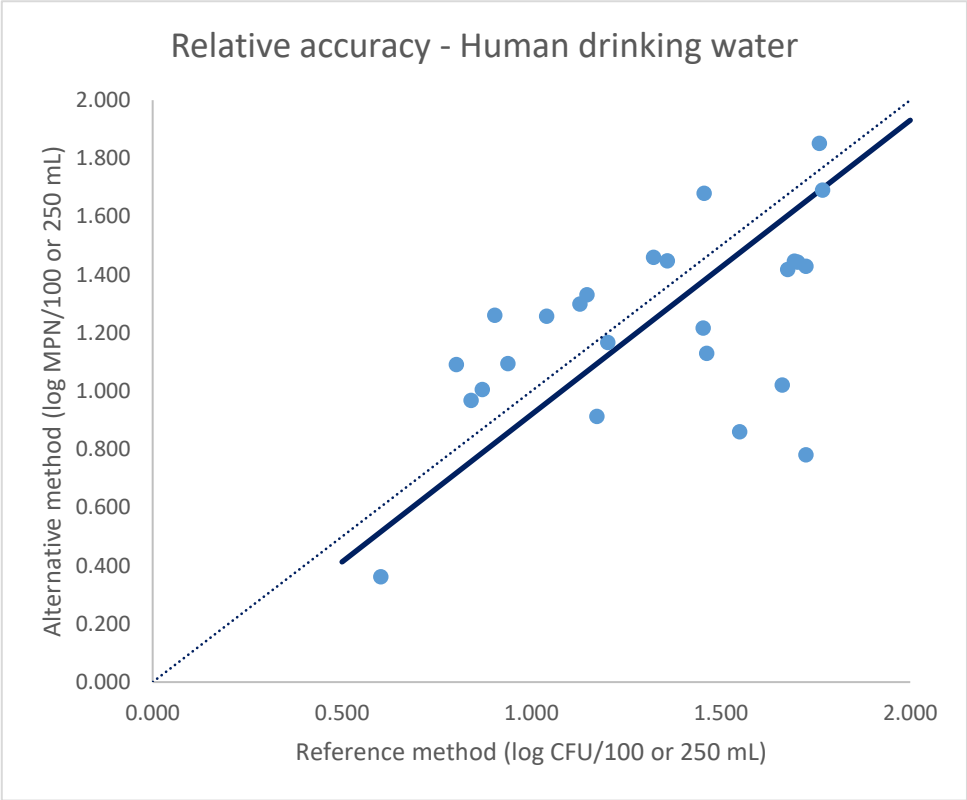
S(b)= 0.910
S(a)= 1.259

t(b)= 0.013
t(a)= 0.073

-2.060**<0.013**<2.060 **Hypothèse b=1 validée**
-2.060**<0.073**<2.060 **Hypothèse a=0 validée**

Est. y	Dév.
1.380	0.299
1.630	-0.186
0.758	0.210
1.281	0.166
1.093	-0.180
1.621	-0.175
0.821	0.439
0.718	0.373
1.048	0.251
0.788	0.218
0.517	-0.154
1.067	0.264
1.245	0.215
1.687	0.164
1.696	-0.005
0.959	0.299
1.387	-0.257
1.475	-0.615
1.589	-0.568
1.603	-0.186
1.690	0.398
1.652	-0.223
1.652	-0.870
1.377	-0.160
0.856	0.238
1.122	0.045

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Raw results for pool water category

Number	Sample	Strain	Level (CFU/100mL)	Alternative method						Reference method										
				Replicate 1			Replicate 2			Replicate 1					Replicate 2					
				Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)	
1	Pool water 1	PSE 1.13	42	29	42.9	1.632	27	38.4	1.584	71			71	1.851	69			69	1.839	
2	Pool water 2	PSE 1.20	19	8	8.7	0.940	13	15	1.176	47			47	1.672	43			43	1.633	
56	Pool water 3	PSE 1.12	30	0	<1	<1	0	<1	<1		20	100%	20	1.301	20			20	1.301	
57	Pool water 4	PSE 1.12	111	0	<1	<1	0	<1	<1		78	100%	78	1.892	118			118	2.072	
58	Pool water 5	PSE 1.24	210	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
59	Pool water 6	PSE 1.24	>300	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
60	Pool water 7	PSE 1.28	32	28	40.6	1.609	23	30.6	1.486		27	100%	27	1.431		18	100%	18	1.255	
61	Pool water 8	PSE 1.28	65	40	78.2	1.893	41	83.1	1.920		61	100%	61	1.785		65	100%	65	1.813	
62	Pool water 9	PSE 1.6	10	24	32.4	1.511	20	25.4	1.405		34	100%	34	1.531		40	100%	40	1.602	
63	Pool water 10	PSE 1.6	51	47	129.8	2.113	49	165.2	2.218		146	100%	146	2.164		124	100%	124	2.093	
64	Pool water 11	PSE 1.24	59	40	78.2	1.893	38	69.7	1.843	95			95	1.978	32			32	1.505	
65	Pool water 12	PSE 1.24	87	48	144.5	2.160	47	129.8	2.113	115			115	2.061	82			82	1.914	
66	Pool water 13	PSE 1.28	148	48	144.5	2.160	51	>200.5	>2.302		131	100%	131	2.117		129	100%	129	2.111	
67	Pool water 14	PSE 1.28	217	51	>200.5	>2.302	49	165.2	2.218		227	100%	227	2.356		172	100%	172	2.236	
68	Pool water 15	PSE 1.12	>300	0	<1	<1	0	<1	<1	>150			>150	>2.176	>150				>2.176	
69	Pool water 16	PSE 1.12	>300	0	<1	<1	0	<1	<1	>150			>150	>2.176	>150				>2.176	
70	Pool water 17	PSE 1.19	30	29	42.9	1.632	38	69.7	1.843		50	100%	50	1.699		59	100%	59	1.771	
71	Pool water 18	PSE 1.19	150	49	165.2	2.218	50	200.5	>2.302		96	100%	96	1.982		151	100%	151	2.179	
72	Pool water 19	PSE 1.6	126	46	118.4	2.073	50	200.5	>2.302		114	100%	114	2.057		105	100%	105	2.021	
73	Pool water 20	PSE 1.6	9	3	3.1	0.491	9	9.9	0.996		3	100%	3	0.477		5	100%	5	0.699	
74	Pool water 21	PSE 1.24	100	45	109.1	2.038	46	118.4	2.073		195	100%	195	2.290		92	100%	92	1.964	
75	Pool water 22	PSE 1.24	77	48	114.5	2.059	49	165.2	2.218		116	100%	116	2.064		75	100%	75	1.875	
76	Pool water 23	PSE 1.5	155	51	>200.5	>2.302	50	200.5	2.302		>150		>150	>2.176		>150		>150	>2.176	
77	Pool water 24	PSE 1.5	302	51	>200.5	>2.302	51	>200.5	>2.302		>150		>150	>2.176		>150		>150	>2.176	
78	Pool water 25	PSE 1.7	113	51	>200.5	>2.302	51	>200.5	>2.302		>150		>150	>2.176		>150		>150	>2.176	
79	Pool water 26	PSE 1.7	86	51	>200.5	>2.302	50	200.5	2.302		>150		>150	>2.176		>150		>150	>2.176	
80	Pool water 27	PSE 1.14	157	46	118.4	2.073	47	129.8	2.113	93			93	1.968	119			119	2.076	
81	Pool water 28	PSE 1.14	117	50	200.5	2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
82	Pool water 29	PSE 1.16	174	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
83	Pool water 30	PSE 1.16	154	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
84	Pool water 31	PSE 1.5	20	8	8.7	0.940	19	23.8	1.377		20	100%	20	1.301		13	100%	13	1.114	
85	Pool water 32	PSE 1.5	50	32	50.4	1.702	33	52.1	1.717		60	100%	60	1.778		57	100%	57	1.756	
86	Pool water 33	PSE 1.7	55	42	88.5	1.947	41	83.1	1.920		62	100%	62	1.792		40	100%	40	1.602	
87	Pool water 34	PSE 1.7	13	10	11.1	1.045	11	12.4	1.093		7	100%	7	0.845		9	100%	9	0.954	
88	Pool water 35	PSE 1.14	35	35	59.1	1.772	40	78.2	1.893	54			54	1.732	66			66	1.820	
89	Pool water 36	PSE 1.14	21	26	36.4	1.561	21	27.1	1.433	32			32	1.505	29			29	1.462	
90	Pool water 37	PSE 1.16	14	14	16.4	1.215	11	12.4	1.093	8			8	0.903	23			23	1.362	
91	Pool water 38	PSE 1.16	24	22	28.8	1.459	19	23.8	1.377	21			21	1.322	20			20	1.301	
93	Pool water 39	202009-2424	45	31	47.8	1.679	29	42.9	1.632	34			34	1.531	36			36	1.556	
94	Pool water 40	202009-2424	26	23	30.6	1.486	25	34.4	1.537	39			39	1.591	38			38	1.580	
95	Pool water 41	202009-2424	17	15	17.8	1.250	17	20.7	1.316	16			16	1.204	19			19	1.279	
96	Pool water 42	202009-2424	38	29	42.9	1.632	28	40.6	1.609	36			36	1.556	35			35	1.544	
97	Pool water 43	20202009-2477	41	38	69.7	1.843	38	69.7	1.843	53			53	1.724	58			58	1.763	
98	Pool water 44	20202009-2477	68	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	2023
99	Pool water 45	20202009-2477	77	51	>200.5	>2.302	51	>200.5	>2.302	>150			>150	>2.176	>150			>150	>2.176	
100	Pool water 46	202010-241	14	11	12.4	1.093	13	15	1.176	8			8	0.903	5			5	0.699	
101	Pool water 47	202010-241	31	28	47.8	1.679	28	40.6	1.609	31			31	1.491	31			31	1.491	
102	Pool water 48	202010-241	21	9	9.9	0.996	12	13.7	1.137	10			10	1.000	9			9	0.954	
103	Pool water 49	202009-2424	23	12	13.7	1.137	13	15	1.176	12			12	1.079	15			15	1.176	
104	Pool water 50	20202009-2477	32	26	50.4	1.702	24	32.4	1.511	37			37	1.568	33			33	1.519	

Total exploitable results for pool water category

Number	Sample	Strain	Level (CFU/100mL)	Alternative method						Reference method									
				Replicate 1			Replicate 2			Replicate 1					Replicate 2				
				Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Nb of fluo wells	MPN/100mL	LOG (NPP/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)	Blue green colony	Nb of Fluorescent colony (non blue-green)	% of ammonium +	CFU/100mL	LOG (CFU/100mL)
1	Pool water 1	PSE 1.13	42	29	42.9	1.632	27	38.4	1.584	71			71	1.851	69			69	1.839
2	Pool water 2	PSE 1.20	19	8	8.7	0.940	13	15	1.176	47			47	1.672	43			43	1.633
60	Pool water 7	PSE 1.28	32	28	40.6	1.609	23	30.6	1.486		27	100%	27	1.431		18	100%	18	1.255
61	Pool water 8	PSE 1.28	65	40	78.2	1.893	41	83.1	1.920		61	100%	61	1.785		65	100%	65	1.813
62	Pool water 9	PSE 1.6	10	24	32.4	1.511	20	25.4	1.405		34	100%	34	1.531		40	100%	40	1.602
70	Pool water 17	PSE 1.19	30	29	42.9	1.632	38	69.7	1.843		50	100%	50	1.699		59	100%	59	1.771
84	Pool water 31	PSE 1.5	20	8	8.7	0.940	19	23.8	1.377		20	100%	20	1.301		13	100%	13	1.114
85	Pool water 32	PSE 1.5	50	32	50.4	1.702	33	52.1	1.717		60	100%	60	1.778		57	100%	57	1.756
86	Pool water 33	PSE 1.7	55	42	88.5	1.947	41	83.1	1.920		62	100%	62	1.792		40	100%	40	1.602
87	Pool water 34	PSE 1.7	13	10	11.1	1.045	11	12.4	1.093		7	100%	7	0.845		9	100%	9	0.954
88	Pool water 35	PSE 1.14	35	35	59.1	1.772	40	78.2	1.893	54			54	1.732	66			66	1.820
89	Pool water 36	PSE 1.14	21	26	36.4	1.561	21	27.1	1.433	32			32	1.505	29			29	1.462
90	Pool water 37	PSE 1.16	14	14	16.4	1.215	11	12.4	1.093	8			8	0.903	23			23	1.362
91	Pool water 38	PSE 1.16	24	22	28.8	1.459	19	23.8	1.377	21			21	1.322	20			20	1.301
93	Pool water 39	202009-2424	45	31	47.8	1.679	29	42.9	1.632	34			34	1.531	36			36	1.556
94	Pool water 40	202009-2424	26	23	30.6	1.486	25	34.4	1.537	39			39	1.591	38			38	1.580
95	Pool water 41	202009-2424	17	15	17.8	1.250	17	20.7	1.316	16			16	1.204	19			19	1.279
96	Pool water 42	202009-2424	38	29	42.9	1.632	28	40.6	1.609	36			36	1.556	35			35	1.544
97	Pool water 43	20202009-2477	41	38	69.7	1.843	38	69.7	1.843	53			53	1.724	58			58	1.763
100	Pool water 46	202010-241	14	11	12.4	1.093	13	15	1.176	8			8	0.903	5			5	0.699
101	Pool water 47	202010-241	31	28	47.8	1.679	28	40.6	1.609	31			31	1.491	31			31	1.491
102	Pool water 48	202010-241	21	9	9.9	0.996	12	13.7	1.137	10			10	1.000	9			9	0.954
103	Pool water 49	202009-2424	23	12	13.7	1.137	13	15	1.176	12			12	1.079	15			15	1.176
104	Pool water 50	20202009-2477	32	26	50.4	1.702	24	32.4	1.511	37			37	1.568	33			33	1.519

Relative accuracy – Pool water – CFU/ 100 mL

Méthode de référence					Méthode alternative					
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	Différence
1	71	69	70.0	1.4	1	42.9	38.4	40.7	3.2	-29.4
2	47	43	45.0	2.8	2	8.7	15.0	11.9	4.5	-33.2
3	27	18	22.5	6.4	3	40.6	30.6	35.6	7.1	13.1
4	61	65	63.0	2.8	4	78.2	83.1	80.7	3.5	17.7
5	34	40	37.0	4.2	5	32.4	25.4	28.9	4.9	-8.1
6	50	59	54.5	6.4	6	42.9	69.7	56.3	19.0	1.8
7	20	13	16.5	4.9	7	8.7	23.8	16.3	10.7	-0.3
8	60	57	58.5	2.1	8	50.4	52.1	51.3	1.2	-7.3
9	62	40	51.0	15.6	9	88.5	83.1	85.8	3.8	34.8
10	7	9	8.0	1.4	10	11.1	12.4	11.8	0.9	3.8
11	54	66	60.0	8.5	11	59.1	78.2	68.7	13.5	8.7
12	32	29	30.5	2.1	12	36.4	27.1	31.8	6.6	1.3
13	8	23	15.5	10.6	13	16.4	12.4	14.4	2.8	-1.1
14	21	20	20.5	0.7	14	28.8	23.8	26.3	3.5	5.8
15	34	36	35.0	1.4	15	47.8	42.9	45.4	3.5	10.4
16	39	38	38.5	0.7	16	30.6	34.4	32.5	2.7	-6.0
17	16	19	17.5	2.1	17	17.8	20.7	19.3	2.1	1.8
18	36	35	35.5	0.7	18	42.9	40.6	41.8	1.6	6.3
19	53	58	55.5	3.5	19	69.7	69.7	69.7	0.0	14.2
20	8	5	6.5	2.1	20	12.4	15.0	13.7	1.8	7.2
21	31	31	31.0	0.0	21	47.8	40.6	44.2	5.1	13.2
22	10	9	9.5	0.7	22	9.9	13.7	11.8	2.7	2.3
23	12	15	13.5	2.1	23	13.7	15.0	14.4	0.9	0.9
24	37	33	35.0	2.8	24	50.4	32.4	41.4	12.7	6.4

q= 24
n= 2
N=qn= 48

Mx= 34.583
MEDx= 35.000
SDbx= 19.156

MEDwx= 2.121
SDwx= 3.653
rob.SDwx= 3.145

My= 37.254
MEDy= 34.050
SDby= 22.415

MEDwy= 3.465
SDwy= 4.642
rob.SDwy= 5.137

M= 2.671
MED= 3.025

Choix de la méthode

GMFR

R= 1.271
rob.R= 1.633

Sx= 19.295
Sy= 22.686

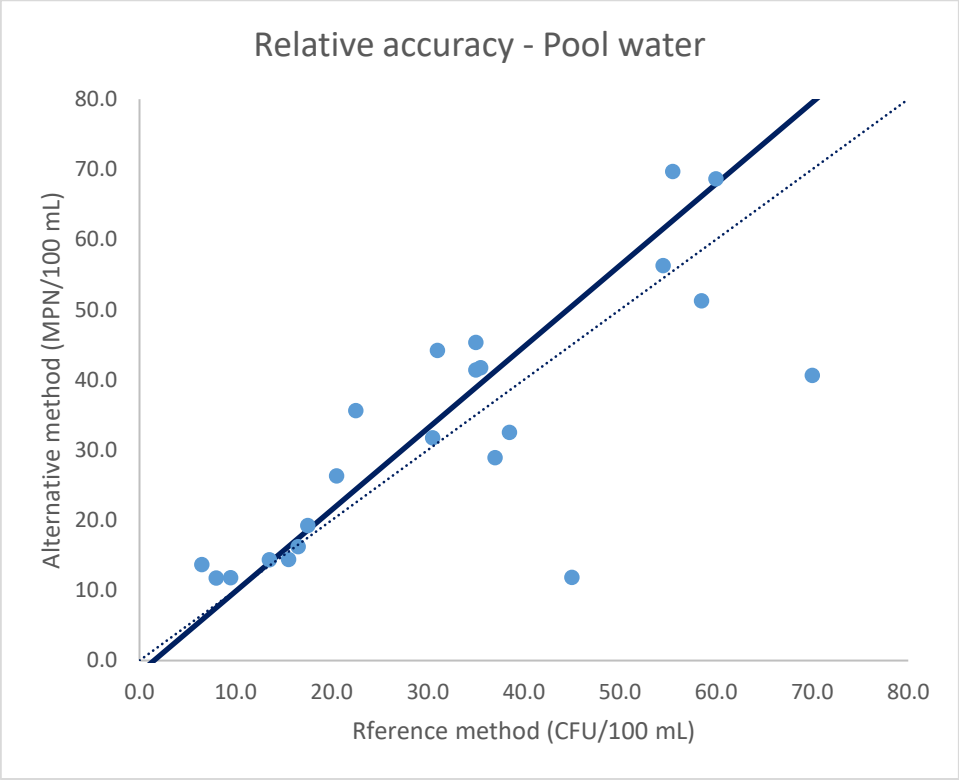
r= 0.775
b= 1.176
a= -3.272

Res. SEM= 69.739
Res.SD= 98.625

S(b)= 0.754 **t(b)=** 0.233 -2.064**<0.233**<2.064 **Hypothèse b=1 validée**
S(a)= 29.698 **t(a)=** 0.110 -2.064**<0.110**<2.064 **Hypothèse a=0 validée**

Est. y	Dév.
79.0	-38.4
49.6	-37.8
23.2	12.4
70.8	9.8
40.2	-11.3
60.8	-4.5
16.1	0.1
65.5	-14.3
56.7	29.1
6.1	5.6
67.3	1.4
32.6	-0.8
15.0	-0.6
20.8	5.5
37.9	7.5
42.0	-9.5
17.3	1.9
38.5	3.3
62.0	7.7
4.4	9.3
33.2	11.0
7.9	3.9
12.6	1.7
37.9	3.5

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Relative accuracy – Pool water – log CFU/ 100 mL

Méthode de référence				
Echantillon	Répétition 1	Répétition 2	M	SD
1	1.851	1.839	1.845	0.009
2	1.672	1.633	1.653	0.027
3	1.431	1.255	1.343	0.125
4	1.785	1.813	1.799	0.020
5	1.531	1.602	1.567	0.050
6	1.699	1.771	1.735	0.051
7	1.301	1.114	1.207	0.132
8	1.778	1.756	1.767	0.016
9	1.792	1.602	1.697	0.135
10	0.845	0.954	0.900	0.077
11	1.732	1.820	1.776	0.062
12	1.505	1.462	1.484	0.030
13	0.903	1.362	1.132	0.324
14	1.322	1.301	1.312	0.015
15	1.531	1.556	1.544	0.018
16	1.591	1.580	1.585	0.008
17	1.204	1.279	1.241	0.053
18	1.556	1.544	1.550	0.009
19	1.724	1.763	1.744	0.028
20	0.903	0.699	0.801	0.144
21	1.491	1.491	1.491	0.000
22	1.000	0.954	0.977	0.032
23	1.079	1.176	1.128	0.069
24	1.568	1.519	1.543	0.035

Méthode alternative				
Echantillon	Répétition 1	Répétition 2	M	SD
1	1.632	1.584	1.608	0.034
2	0.940	1.176	1.058	0.167
3	1.609	1.486	1.547	0.087
4	1.893	1.920	1.906	0.019
5	1.511	1.405	1.458	0.075
6	1.632	1.843	1.738	0.149
7	0.940	1.377	1.158	0.309
8	1.702	1.717	1.710	0.010
9	1.947	1.920	1.933	0.019
10	1.045	1.093	1.069	0.034
11	1.772	1.893	1.832	0.086
12	1.561	1.433	1.497	0.091
13	1.215	1.093	1.154	0.086
14	1.459	1.377	1.418	0.059
15	1.679	1.632	1.656	0.033
16	1.486	1.537	1.511	0.036
17	1.250	1.316	1.283	0.046
18	1.632	1.609	1.620	0.017
19	1.843	1.843	1.843	0.000
20	1.093	1.176	1.135	0.058
21	1.679	1.609	1.644	0.050
22	0.996	1.137	1.066	0.100
23	1.137	1.176	1.156	0.028
24	1.702	1.511	1.606	0.136

Différence
-0.237
-0.595
0.204
0.107
-0.109
0.003
-0.049
-0.057
0.236
0.170
0.056
0.013
0.022
0.106
0.112
-0.074
0.042
0.070
0.099
0.334
0.153
0.089
0.029
0.063

q= 24
n= 2
N=qn= 48

Mx= 1.451
MEDx= 1.544
SDbx= 0.302

MEDwx= 0.034
SDwx= 0.071
rob.SDwx= 0.050

My= 1.484
MEDy= 1.529
SDby= 0.285

MEDwy= 0.054
SDwy= 0.067
rob.SDwy= 0.080

M= 0.033
MED= 0.060

Choix de la méthode

GMFR

R= 0.946
rob.R= 1.609

Sx= 0.306
Sy= 0.291

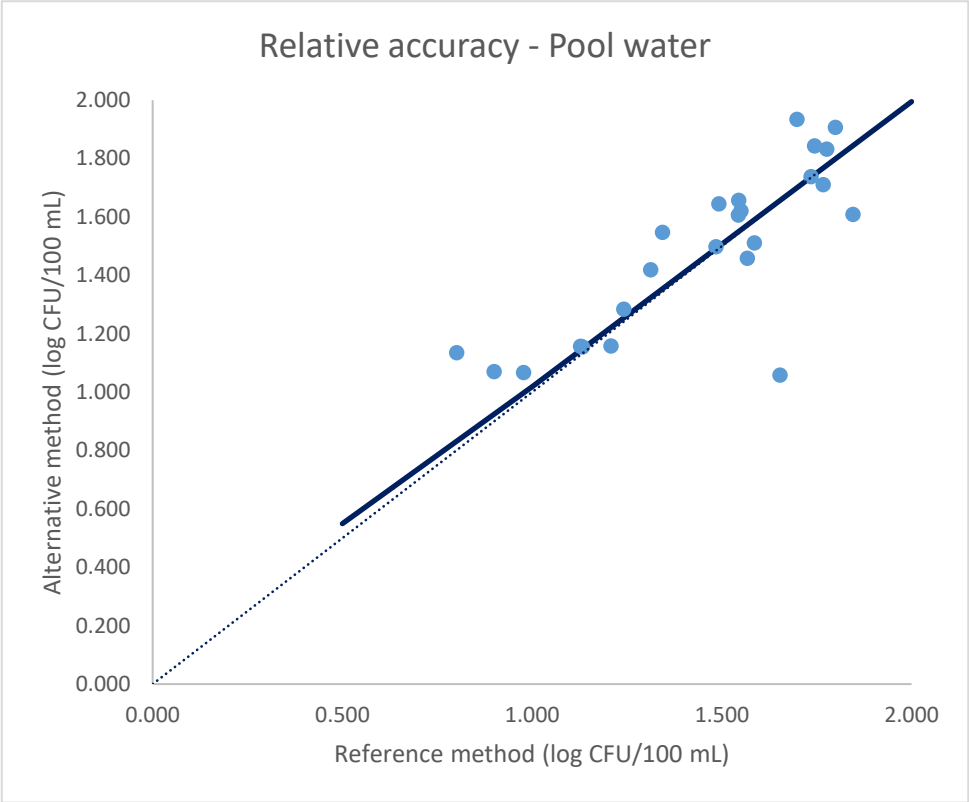
r= 0.790
b= 0.949
a= 0.106

Res. SEM= 0.832
Res.SD= 1.176

S(b)= 0.566 **t(b)=** 0.089 -2.064**<0.089**<2.064 **Hypothèse b=1 validée**
S(a)= 0.839 **t(a)=** 0.127 -2.064**<0.127**<2.064 **Hypothèse a=0 validée**

Est. y	Dév.
1.858	-0.249
1.675	-0.618
1.382	0.166
1.814	0.092
1.594	-0.136
1.753	-0.015
1.253	-0.095
1.784	-0.074
1.718	0.216
0.960	0.109
1.792	0.040
1.515	-0.018
1.181	-0.027
1.351	0.067
1.572	0.084
1.611	-0.100
1.285	-0.002
1.578	0.043
1.762	0.081
0.867	0.268
1.522	0.122
1.034	0.032
1.177	-0.020
1.571	0.035

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Relative accuracy – All category – CFU/ 100 or 250 mL

Méthode de référence					Méthode alternative					
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	Différence
1	24	34	29.0	7.1	1	45.3	50.4	47.9	3.6	18.9
2	54	47	50.5	4.9	2	32.4	23.8	28.1	6.1	-22.4
3	6	8	7.0	1.4	3	9.9	8.7	9.3	0.8	2.3
4	20	26	23.0	4.2	4	25.6	30.7	28.2	3.6	5.2
5	17	13	15.0	2.8	5	7.2	9.3	8.3	1.5	-6.8
6	47	52	49.5	3.5	6	27.1	28.8	28.0	1.2	-21.6
7	8	8	8.0	0.0	7	19.5	17.0	18.3	1.8	10.3
8	4	10	7.0	4.2	8	11.1	13.7	12.4	1.8	5.4
9	15	12	13.5	2.1	9	19.2	20.7	20.0	1.1	6.5
10	11	5	8.0	4.2	10	7.5	13.7	10.6	4.4	2.6
11	4	4	4.0	0.0	11	5.3	1.0	3.2	3.0	-0.9
12	14	14	14.0	0.0	12	20.7	22.2	21.5	1.1	7.5
13	20	22	21.0	1.4	13	27.1	30.6	28.9	2.5	7.9
14	59	56	57.5	2.1	14	75.1	67.1	71.1	5.7	13.6
15	66	52	59.0	9.9	15	34.8	69.2	52.0	24.3	-7.0
16	15	8	11.5	4.9	16	18.1	18.1	18.1	0.0	6.6
17	28	30	29.0	1.4	17	11.1	16.4	13.8	3.7	-15.3
18	33	38	35.5	3.5	18	5.3	9.9	7.6	3.3	-27.9
19	43	49	46.0	4.2	19	9.9	11.1	10.5	0.8	-35.5
20	46	49	47.5	2.1	20	27.8	24.6	26.2	2.3	-21.3
21	54	62	58.0	5.7	21	185.0	81.2	133.1	73.4	75.1
22	52	54	53.0	1.4	22	22.2	32.4	27.3	7.2	-25.7
23	55	51	53.0	2.8	23	8.7	4.2	6.5	3.2	-46.6
24	23	35	29.0	8.5	24	20.9	13.0	17.0	5.6	-12.1
25	5	15	10.0	7.1	25	9.3	16.6	13.0	5.2	3.0
26	18	14	16.0	2.8	26	21	10	15.6	7.4	-0.4
27	71	69	70.0	1.4	27	42.9	38.4	40.7	3.2	-29.4
28	47	43	45.0	2.8	28	8.7	15.0	11.9	4.5	-33.2
29	27	18	22.5	6.4	29	40.6	30.6	35.6	7.1	13.1
30	61	65	63.0	2.8	30	78.2	83.1	80.7	3.5	17.7
31	34	40	37.0	4.2	31	32.4	25.4	28.9	4.9	-8.1
32	50	59	54.5	6.4	32	42.9	69.7	56.3	19.0	1.8
33	20	13	16.5	4.9	33	8.7	23.8	16.3	10.7	-0.3
34	60	57	58.5	2.1	34	50.4	52.1	51.3	1.2	-7.3
35	62	40	51.0	15.6	35	88.5	83.1	85.8	3.8	34.8
36	7	9	8.0	1.4	36	11.1	12.4	11.8	0.9	3.8
37	54	66	60.0	8.5	37	59.1	78.2	68.7	13.5	8.7
38	32	29	30.5	2.1	38	36.4	27.1	31.8	6.6	1.3
39	8	23	15.5	10.6	39	16.4	12.4	14.4	2.8	-1.1
40	21	20	20.5	0.7	40	28.8	23.8	26.3	3.5	5.8
41	34	36	35.0	1.4	41	47.8	42.9	45.4	3.5	10.4
42	39	38	38.5	0.7	42	30.6	34.4	32.5	2.7	-6.0
43	16	19	17.5	2.1	43	17.8	20.7	19.3	2.1	1.8
44	36	35	35.5	0.7	44	42.9	40.6	41.8	1.6	6.3
45	53	58	55.5	3.5	45	69.7	69.7	69.7	0.0	14.2
46	8	5	6.5	2.1	46	12.4	15.0	13.7	1.8	7.2
47	31	31	31.0	0.0	47	47.8	40.6	44.2	5.1	13.2
48	10	9	9.5	0.7	48	9.9	13.7	11.8	2.7	2.3
49	12	15	13.5	2.1	49	13.7	15.0	14.4	0.9	0.9
50	37	33	35.0	2.8	50	50.4	32.4	41.4	12.7	6.4

q= 50
n= 2
N=qn= 100

Mx= 31.690
MEDx= 29.750
SDbx= 19.252

My= 31.399
MEDy= 26.250
SDby= 25.156

M= -0.291
MED= 2.300

MEDwx= 2.828
SDwx= 3.098
rob.SDwx= 4.193

MEDwy= 3.359
SDwy= 10.777
rob.SDwy= 4.980

Est. y	Dév.
22.9	24.9
52.2	-24.1
-7.0	16.3
14.8	13.4
3.9	4.4
50.9	-22.9
-5.7	23.9
-7.0	19.4
1.8	18.1
-5.7	16.3
-11.1	14.2
2.5	18.9
12.1	16.8
61.8	9.3
63.8	-11.8
-0.9	19.0
22.9	-9.2
31.8	-24.2
46.1	-35.6
48.1	-21.9
62.4	70.7
55.6	-28.3
55.6	-49.2
22.9	-6.0
-2.9	15.9
5.2	10.4
78.8	-38.1
44.7	-32.9
14.1	21.5
69.2	11.4
33.8	-4.9
57.7	-1.4
5.9	10.3
63.1	-11.9
52.9	32.9
-5.7	17.4
65.2	3.5
25.0	6.8
4.6	9.8
11.4	14.9
31.1	14.2
35.9	-3.4
7.3	12.0
31.8	10.0
59.0	10.7
-7.7	21.4
25.7	18.5
-3.6	15.4
1.8	12.5
31.1	10.3

Choix de la méthode
GMFR

R= 3.478
rob.R= 1.188

Sx= 19.145
Sy= 26.480

r= 0.620
b= 1.362
a= -16.547

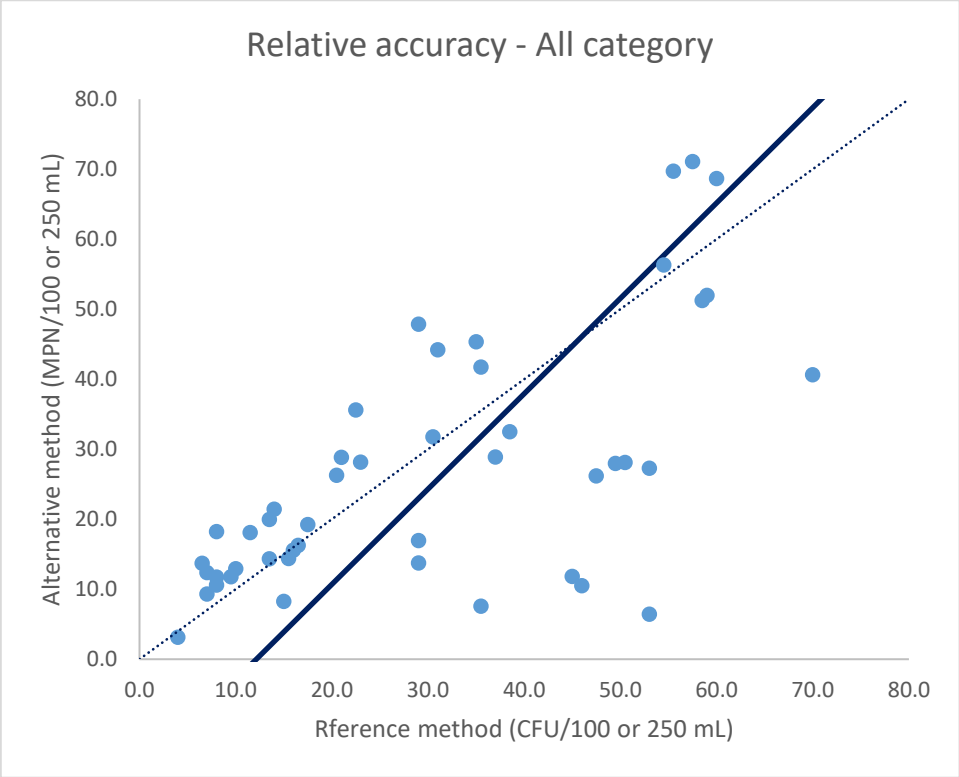
Res. SEM= 127.455
Res.SD= 180.248

S(b)= 0.936
S(a)= 34.720

t(b)= 0.386
t(a)= 0.477

-2.001**<0.386**<2.001 **Hypothèse b=1 validée**
-2.001**<0.477**<2.001 **Hypothèse a=0 validée**

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Relative accuracy – All category – log CFU/ 100 or 250 mL

Méthode de référence					Méthode alternative					
Echantillon	Répétition 1	Répétition 2	M	SD	Echantillon	Répétition 1	Répétition 2	M	SD	Différence
1	1.380	1.531	1.456	0.107	1	1.656	1.702	1.679	0.033	0.223
2	1.732	1.672	1.702	0.043	2	1.511	1.377	1.444	0.095	-0.259
3	0.778	0.903	0.841	0.088	3	0.996	0.940	0.968	0.040	0.127
4	1.301	1.415	1.358	0.081	4	1.408	1.487	1.448	0.056	0.090
5	1.230	1.114	1.172	0.082	5	0.857	0.968	0.913	0.079	-0.259
6	1.672	1.716	1.694	0.031	6	1.433	1.459	1.446	0.019	-0.248
7	0.903	0.903	0.903	0.000	7	1.290	1.230	1.260	0.042	0.357
8	0.602	1.000	0.801	0.281	8	1.045	1.137	1.091	0.065	0.290
9	1.176	1.079	1.128	0.069	9	1.283	1.316	1.300	0.023	0.172
10	1.041	0.699	0.870	0.242	10	0.875	1.137	1.006	0.185	0.136
11	0.602	0.602	0.602	0.000	11	0.724	0.000	0.362	0.512	-0.240
12	1.146	1.146	1.146	0.000	12	1.316	1.346	1.331	0.021	0.185
13	1.301	1.342	1.322	0.029	13	1.433	1.486	1.459	0.037	0.138
14	1.771	1.748	1.760	0.016	14	1.876	1.827	1.851	0.035	0.092
15	1.820	1.716	1.768	0.073	15	1.542	1.840	1.691	0.211	-0.077
16	1.176	0.903	1.040	0.193	16	1.258	1.258	1.258	0.000	0.218
17	1.447	1.477	1.462	0.021	17	1.045	1.215	1.130	0.120	-0.332
18	1.519	1.580	1.549	0.043	18	0.724	0.996	0.860	0.192	-0.689
19	1.633	1.690	1.662	0.040	19	0.996	1.045	1.020	0.035	-0.641
20	1.663	1.690	1.676	0.019	20	1.444	1.391	1.417	0.038	-0.259
21	1.732	1.792	1.762	0.042	21	2.267	1.910	2.088	0.253	0.326
22	1.716	1.732	1.724	0.012	22	1.346	1.511	1.428	0.116	-0.296
23	1.740	1.708	1.724	0.023	23	0.940	0.623	0.781	0.224	-0.943
24	1.362	1.544	1.453	0.129	24	1.320	1.114	1.217	0.146	-0.236
25	0.699	1.176	0.938	0.337	25	0.968	1.220	1.094	0.178	0.157
26	1.255	1.146	1.201	0.077	26	1.318	1.017	1.168	0.213	-0.033
27	1.851	1.839	1.845	0.009	27	1.632	1.584	1.608	0.034	-0.237
28	1.672	1.633	1.653	0.027	28	0.940	1.176	1.058	0.167	-0.595
29	1.431	1.255	1.343	0.125	29	1.609	1.486	1.547	0.087	0.204
30	1.785	1.813	1.799	0.020	30	1.893	1.920	1.906	0.019	0.107
31	1.531	1.602	1.567	0.050	31	1.511	1.405	1.458	0.075	-0.109
32	1.699	1.771	1.735	0.051	32	1.632	1.843	1.738	0.149	0.003
33	1.301	1.114	1.207	0.132	33	0.940	1.377	1.158	0.309	-0.049
34	1.778	1.756	1.767	0.016	34	1.702	1.717	1.710	0.010	-0.057
35	1.792	1.602	1.697	0.135	35	1.947	1.920	1.933	0.019	0.236
36	0.845	0.954	0.900	0.077	36	1.045	1.093	1.069	0.034	0.170
37	1.732	1.820	1.776	0.062	37	1.772	1.893	1.832	0.086	0.056
38	1.505	1.462	1.484	0.030	38	1.561	1.433	1.497	0.091	0.013
39	0.903	1.362	1.132	0.324	39	1.215	1.093	1.154	0.086	0.022
40	1.322	1.301	1.312	0.015	40	1.459	1.377	1.418	0.059	0.106
41	1.531	1.556	1.544	0.018	41	1.679	1.632	1.656	0.033	0.112
42	1.591	1.580	1.585	0.008	42	1.486	1.537	1.511	0.036	-0.074
43	1.204	1.279	1.241	0.053	43	1.250	1.316	1.283	0.046	0.042
44	1.556	1.544	1.550	0.009	44	1.632	1.609	1.620	0.017	0.070
45	1.724	1.763	1.744	0.028	45	1.843	1.843	1.843	0.000	0.099
46	0.903	0.699	0.801	0.144	46	1.093	1.176	1.135	0.058	0.334
47	1.491	1.491	1.491	0.000	47	1.679	1.609	1.644	0.050	0.153
48	1.000	0.954	0.977	0.032	48	0.996	1.137	1.066	0.100	0.089
49	1.079	1.176	1.128	0.069	49	1.137	1.176	1.156	0.028	0.029
50	1.568	1.519	1.543	0.035	50	1.702	1.511	1.606	0.136	0.063

q= 50
n= 2
N=qn= 100

Mx= 1.391
MEDx= 1.473
SDbx= 0.336

My= 1.366
MEDy= 1.418
SDby= 0.342

M= -0.024
MED= 0.060

MEDwx= 0.043
SDwx= 0.081
rob.SDwx= 0.063

MEDwy= 0.059
SDwy= 0.095
rob.SDwy= 0.087

Est. y	Dév.
1.2	0.5
1.5	0.0
0.6	0.4
1.1	0.3
0.9	0.0
1.5	0.0
0.7	0.6
0.6	0.5
0.9	0.4
0.6	0.4
0.3	0.0
0.9	0.4
1.1	0.4
1.5	0.3
1.5	0.1
0.8	0.5
1.2	-0.1
1.3	-0.5
1.4	-0.4
1.5	0.0
1.5	0.5
1.5	-0.1
1.5	-0.7
1.2	0.0
0.7	0.4
1.0	0.2
1.6	0.0
1.4	-0.4
1.1	0.4
1.6	0.3
1.3	0.1
1.5	0.2
1.0	0.2
1.5	0.2
1.5	0.5
0.7	0.4
1.6	0.3
1.3	0.2
0.9	0.3
1.1	0.3
1.3	0.3
1.4	0.2
1.0	0.3
1.3	0.3
1.5	0.3
0.6	0.6
1.3	0.4
0.7	0.3
0.9	0.3
1.3	0.3

Choix de la méthode
GMFR

R= 1.174
rob.R= 1.376

Sx= 0.343
Sy= 0.353

r= 0.640
b= 1.028
a= -0.272

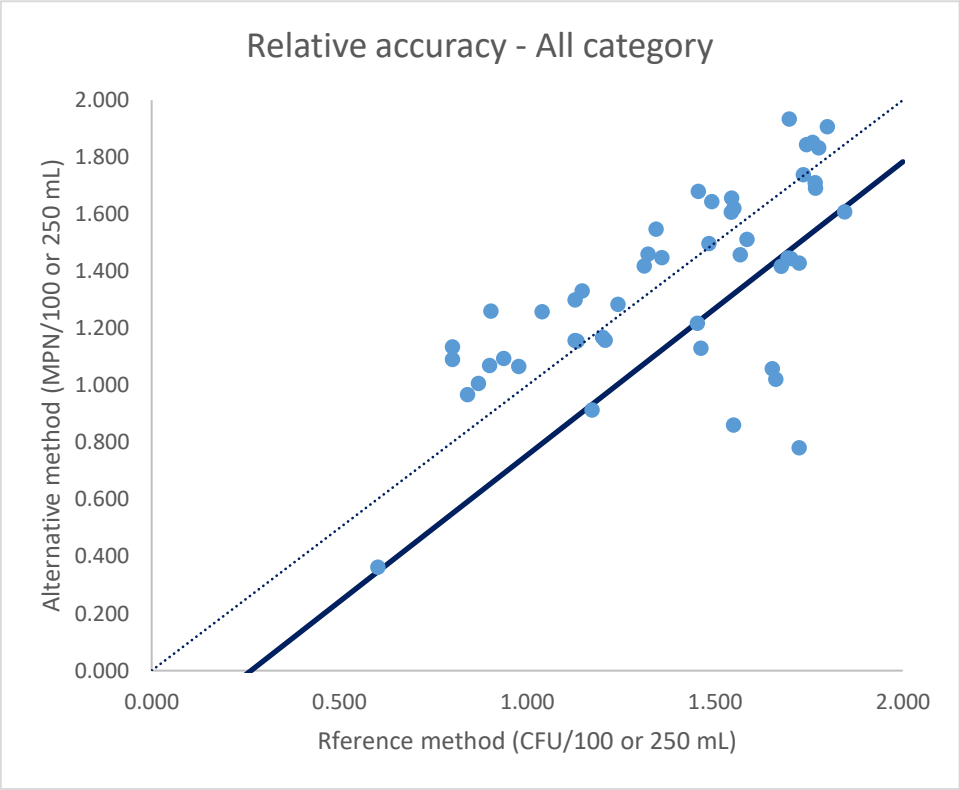
Res. SEM= 3.116
Res.SD= 4.407

S(b)= 1.298
S(a)= 1.858

t(b)= 0.022
t(a)= 0.146

-2.001**<0.022**<2.001 **Hypothèse b=1 validée**
-2.001**<0.146**<2.001 **Hypothèse a=0 validée**

Les points représentés correspondent aux moyennes des répétitions de chaque échantillon



Appendix 5: linearity results

PSEUDALERT - LINEARITY - POOL WATER

N°	Sample	Strain	Target contamination level (CFU/100 mL)	Real contamination level (CFU/100 mL)	Alternative method						Reference method (*)							
					R1			R2			R1				R2			
					Positive wells	MPN/100 mL	log	Positive wells	MPN/100 mL	log	Nb of fluorescent colonies (non blue-green)	% of ammonium +	CFU/100mL	Log (CFU / 100 mL)	Nb of fluorescent colonies (non blue-green)	% of ammonium +	CFU/100mL	Log (CFU / 100 mL)
A	EPSC	PSE 1.28	5	12	9	9,9	0,996	11	12,4	1,093	19	100%	19	1,279	13	100%	13	1,114
B	EPSC	PSE 1.28	15	26	19	23,8	1,377	12	13,7	1,137	22	100%	22	1,342	30	100%	30	1,477
C	EPSC	PSE 1.28	60	66	31	47,8	1,679	36	62,4	1,795	50	100%	50	1,699	61	100%	61	1,785
D	EPSC	PSE 1.28	80	81	39	73,8	1,868	30	45,3	1,656	68	100%	68	1,833	60	100%	60	1,778
E	EPSC	PSE 1.28	100	99	35	59,1	1,772	34	56	1,748	80	100%	80	1,903	97	100%	97	1,987
F	EPSC	PSE 1.28	150	156	43	94,5	1,975	46	118,4	2,073	160	100%	160	2,204	135	100%	135	2,130

Linearity - Pseudalert - Pool water

Level	Reference method Rep.1	Rep.2	M	SD	Alternative method Rep.1	Rep.2	M	SD
1	19	13	16,0	4,243	9,9	12,4	11,2	1,768
2	22	30	26,0	5,657	23,8	13,7	18,8	7,142
3	50	61	55,5	7,778	47,8	62,4	55,1	10,324
4	68	60	64,0	5,657	73,8	45,3	59,6	20,153
5	80	97	88,5	12,021	59,1	56,0	57,6	2,192
6	160	135	147,5	17,678	94,5	118,4	106,5	16,900

q = 6
n = 2
N = qn = 12

Mx = 66,250
MEDx = 59,750
SDbx = 47,670

MEDwx = 6,718
SDwx = 7,068
rob. SDwx = 9,959

My = 51,425
MEDy = 56,325
SDby = 34,160

MEDwy = 8,733
SDwy = 8,452
rob. SDwy = 12,947

Choix méthode

GMFR

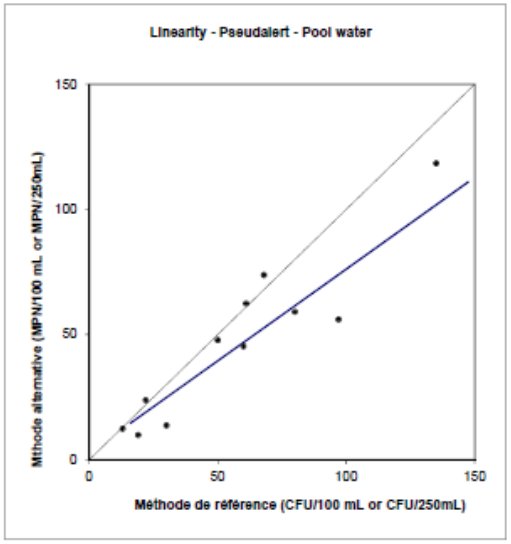
R = 1,196
rob.R = 1,300
Res.SEM = 9,416
Res.SD = 13,316

Sx = 46,048
Sy = 33,745

r = 0,969
b = 0,733
a = 2,875

Est y	Déviation
14,600	-3,450
21,928	-3,178
43,547	11,553
49,776	9,774
67,731	-10,181
110,968	-4,518

Sb = 0,091 **p(t;b=1) =** 0,015 **t (b) =** 2,921
Sa = 7,175 **p(t;a=0) =** 0,697 **t (a) =** 0,401



Linéarité

F = 4,706 **p(F) =** 0,046
rob.F = 1,145 **rob.p(F) =** 0,419

Linearity - Pseudalert - Pool water - Log data

Level	Reference method Rep.1	Rep.2	M	SD	Alternative method Rep.1	Rep.2	M	SD
1	1,279	1,114	1,2	0,117	0,996	1,093	1,0	0,069
2	1,342	1,477	1,4	0,095	1,377	1,137	1,3	0,170
3	1,699	1,785	1,7	0,061	1,679	1,795	1,7	0,082
4	1,833	1,778	1,8	0,038	1,868	1,656	1,8	0,150
5	1,903	1,987	1,9	0,059	1,772	1,748	1,8	0,017
6	2,204	2,130	2,2	0,052	1,975	2,073	2,0	0,069

q = 6
n = 2
N = qn = 12

Mx = 1,711
MEDx = 1,774
SDbx = 0,354

MEDwx = 0,060
SDwx = 0,053
rob. SDwx = 0,089

My = 1,597
MEDy = 1,749
SDby = 0,368

MEDwy = 0,076
SDwy = 0,075
rob. SDwy = 0,112

Choix méthode

GMFR

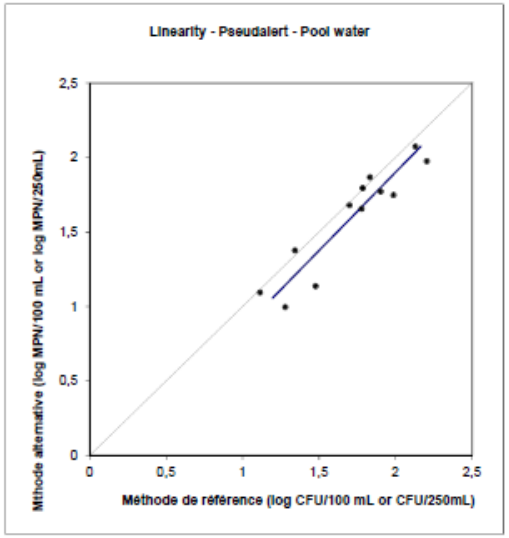
R = 1,410
rob.R = 1,257
Res.SEM = 0,077
Res.SD = 0,108

Sx = 0,343
Sy = 0,360

r = 0,981
b = 1,050
a = -0,198

Est y	Déviation
1,057	-0,013
1,281	-0,025
1,630	0,107
1,697	0,066
1,843	-0,083
2,076	-0,052

Sb = 0,100 **p(t;b=1) =** 0,630 **t (b) =** 0,497
Sa = 0,174 **p(t;a=0) =** 0,280 **t (a) =** 1,141



Linéarité

F = 3,691 **p(F) =** 0,076
rob.F = 0,837 **rob.p(F) =** 0,548

PSEUDALERT - LINEARITY - SPRING WATER

N°	Sample	Strain	Target contamination level (CFU/100 mL)	Real contamination level (CFU/100 mL)	Alternative method						Reference method (*)					
					R1			R2			R1			R2		
					Positive wells	MPN/100 mL	log	Positive wells	MPN/100 mL	log	Blue green colonies	CFU/100mL	Log (CFU / 100 mL)	Blue green colonies	CFU/100mL	Log (CFU / 100 mL)
D	Spring Water	PSE 1.16	5	11	11	12,4	1,093	13	15	1,176	4	4	0,602	14	14	1,146
E	Spring Water	PSE 1.16	15	20	14	16,4	1,215	10	11,1	1,045	15	15	1,176	12	12	1,079
F	Spring Water	PSE 1.16	40	27	18	22,2	1,346	15	17,8	1,250	17	17	1,230	22	22	1,342
G	Spring Water	PSE 1.16	60	34	25	34,4	1,537	18	22,2	1,346	31	31	1,491	18	18	1,255
H	Spring Water	PSE 1.16	80	50	31	47,8	1,679	33	53,1	1,725	34	34	1,531	39	39	1,591
I	Spring Water	PSE 1.16	100	42	34	56	1,748	34	56	1,748	46	46	1,663	21	21	1,322
J	Spring Water	PSE 1.16	150	94	43	94,5	1,975	48	144,5	2,160	67	67	1,826	56	56	1,748

Linearity - Pseudalert - Spring water

Level	Reference method Rep.1	Rep.2	M	SD	Alternative method Rep.1	Rep.2	M	SD
1	4	14	9,0	7,071	12	15	13,7	1,838
2	15	12	13,5	2,121	16	11	13,8	3,748
3	17	22	19,5	3,536	22	18	20,0	3,111
4	31	18	24,5	9,192	34	22	28,3	8,627
5	34	39	36,5	3,536	48	53	50,5	3,748
6	46	21	33,5	17,678	56	56	56,0	0,000
7	67	56	61,5	7,778	95	145	119,5	35,355

q = 7
n = 2
N = qn = 14

Mx = 28,286
MEDx = 24,500
SDbx = 17,701

MEDwx = 7,071
SDwx = 6,193
rob. SDwx = 10,484

My = 43,100
MEDy = 28,300
SDby = 37,718

MEDwy = 3,748
SDwy = 9,876
rob. SDwy = 5,556

Choix méthode
GMFR

R = 1,595
rob.R = 0,530
Res.SEM = 8,257
Res.SD = 11,678

Sx = 18,180
Sy = 37,660

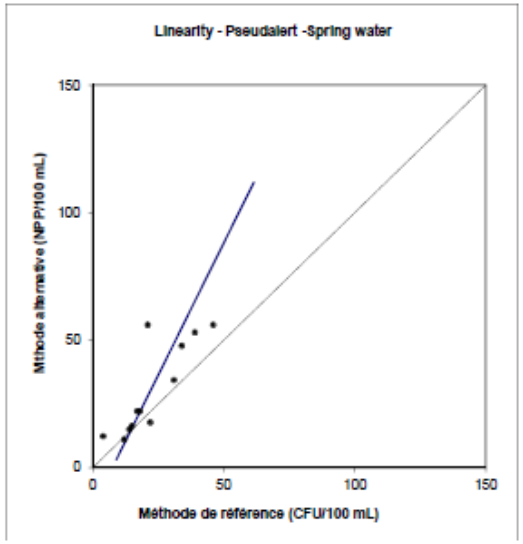
r = 0,980
b = 2,071
a = -15,492

Est y	Déviation
3,151	10,549
12,472	1,278
24,901	-4,901
35,258	-6,958
60,115	-9,665
53,901	2,099
111,901	7,599

Sb = 0,185 **p(t;b=1) =** 0,000 **t (b) =** 5,778
Sa = 6,103 **p(t;a=0) =** 0,026 **t (a) =** 2,538

Linéarité

F = 1,955 **p(F) =** 0,203
rob.F = 9,201 **rob.p(F) =** 0,006



Linearity - Pseudalert - Spring water - Log data

Level	Reference method Rep.1	Rep.2	M	SD	Alternative method Rep.1	Rep.2	M	SD
1	0,602	1,146	0,9	0,385	1,093	1,176	1,1	0,058
2	1,176	1,079	1,1	0,069	1,215	1,045	1,1	0,120
3	1,230	1,342	1,3	0,079	1,346	1,250	1,3	0,068
4	1,491	1,255	1,4	0,167	1,537	1,346	1,4	0,134
5	1,531	1,591	1,6	0,042	1,679	1,725	1,7	0,032
6	1,663	1,322	1,5	0,241	1,748	1,748	1,7	0,000
7	1,826	1,748	1,8	0,055	1,975	2,160	2,1	0,130

q = 7
n = 2
N = qn = 14

Mx = 1,357
MEDx = 1,373
SDbx = 0,299

MEDwx = 0,079
SDwx = 0,134
rob. SDwx = 0,117

My = 1,503
MEDy = 1,441
SDby = 0,351

MEDwy = 0,068
SDwy = 0,065
rob. SDwy = 0,101

Choix méthode
GMFR

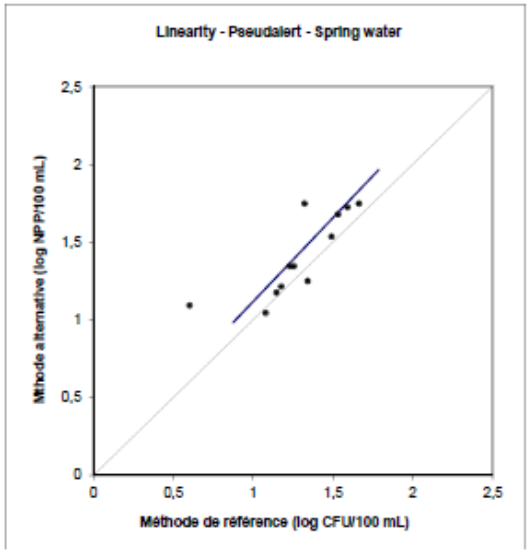
R = 0,484
rob.R = 0,857
Res.SEM = 0,120
Res.SD = 0,170

Sx = 0,319
Sy = 0,344

r = 0,943
b = 1,078
a = 0,040

Est y	Déviation
0,982	0,153
1,255	-0,125
1,427	-0,128
1,520	-0,079
1,723	-0,021
1,649	0,099
1,966	0,101

Sb = 0,154 **p(t;b=1) =** 0,620 **t (b) =** 0,508
Sa = 0,213 **p(t;a=0) =** 0,855 **t (a) =** 0,186



Linéarité

F = 15,133 **p(F) =** 0,001
rob.F = 5,433 **rob.p(F) =** 0,023

Appendix 6: lod-loq results

Limit of detection (LOD) and limit of quantification (LOQ)

Raw results

Strain : *Pseudomonas aeruginosa*

Target level (UFC/ 100mL)	Real level of contamination (UFC/ 100mL) (a)	Replicates					
		1	2	3	4	5	6
		Detection P.aeruginosa (MPN/ 100mL)	Detection P.aeruginosa (MPN/ 100mL)	Detection P.aeruginosa (MPN/ 100mL)	Detection P.aeruginosa (MPN/ 100mL)	Detection P.aeruginosa (MPN/ 100mL)	Detection P.aeruginosa (MPN/ 100mL)
0	0,000	0,0	0,0	0,0	0,0	0,0	0,0
0,3	0,530	0,0	0,0	0,0	0,0	0,0	0,0
0,6	0,600	0,0	0,0	0,0	0,0	0,0	0,0
1	1,500	0,0	0,0	0,0	2,0	0,0	0,0
2	2,100	1,0	4,2	1,0	2,0	0,0	1,0
3	2,800	2,0	2,0	1,0	2,0	4,2	2,0

(a): level calculated from 30 enumerations

Appendix 7: selectivity results

Inclusivity

N°	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of positive wells	Alternative method result (MPN/100 mL)	Reference method (if needed)		
							Number of typical colonies	Confirmation	Result (CFU/100 mL)
1	PSE.1.1	*Pseudomonas aeruginosa*	ATCC 19429	39	2	2	/	/	/
2	PSE.1.3	*Pseudomonas aeruginosa*	CIP 100.720	38	29	42,9	/	/	/
3	PSE.1.4	*Pseudomonas aeruginosa*	Eau de fontaine	50	33	53,1	/	/	/
4	PSE.1.5	*Pseudomonas aeruginosa*	Eau de fontaine	35	45	109,1	/	/	/
5	PSE.1.6	*Pseudomonas aeruginosa*	Eau de fontaine	33	28	40,6	/	/	/
6	PSE.1.8	*Pseudomonas aeruginosa*	Eau de Fontaine	31	24	32,4	/	/	/
7	PSE.1.9	*Pseudomonas aeruginosa*	INRA (code 1059 PPa)	44	33	53,1	/	/	/
8	PSE.1.13	*Pseudomonas aeruginosa*	Eau	38	30	45,3	/	/	/
9	PSE.1.14	*Pseudomonas aeruginosa*	Eau	69	44	101,3	/	/	/
10	PSE.1.15	*Pseudomonas aeruginosa*	Eau	53	37	65,9	/	/	/
11	PSE.1.16	*Pseudomonas aeruginosa*	Eau	76	45	109,1	/	/	/
12	PSE.1.17	*Pseudomonas aeruginosa*	Eau	96	47	129,8	/	/	/
13	PSE.1.18	*Pseudomonas aeruginosa*	Eau	82	43	94,5	/	/	/
14	PSE.1.19	*Pseudomonas aeruginosa*	Eau	39	28	40,6	/	/	/
15	PSE.1.20	*Pseudomonas aeruginosa*	Eau	71	42	88,5	/	/	/
16	PSE.1.21	*Pseudomonas aeruginosa*	Eau	45	34	56	/	/	/
17	PSE.1.22	*Pseudomonas aeruginosa*	Eau	67	40	78,2	/	/	/
18	PSE.1.23	*Pseudomonas aeruginosa*	Eau	90	46	118,4	/	/	/
19	PSE.1.24	*Pseudomonas aeruginosa*	Eau	54	39	73,8	/	/	/
20	PSE.1.25	*Pseudomonas aeruginosa*	Eau	59	32	50,4	/	/	/
21	PSE.1.26	*Pseudomonas aeruginosa*	Eau	48	28	40,6	/	/	/
22	PSE.1.27	*Pseudomonas aeruginosa*	Eau	73	43	94,5	/	/	/
23	PSE.1.28	*Pseudomonas aeruginosa*	ATCC10145	43	24	32,4	/	/	/
24	PSE.1.29	*Pseudomonas aeruginosa*	ATCC 27853	37	36	62,4	/	/	/
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				—	–	—	—	–	—

Exclusivity

N°	Code	Species	Origin	Inoculation level (CFU/100 mL)	Number of positive wells	Result (MPN/100 mL)
1	ACI.1.1	*Acinetobacter baumanii*	sandwich	1,30E+04	0	<1
2	ACI.2.1	*Acinetobacter cloacae*	Eau de la Seine	3,30E+03	0	<1
3	AER.1.4	*Aeromonas hydrophila*	Eau (Japon)	1,50E+04	0	<1
4	AER.1.5	*Aeromonas hydrophila*	Eau de rivière (Saint Oger)	9,30E+03	0	<1
5	AER.1.6	*Aeromonas hydrophila*	Eau de rivière (Orge)	1,30E+04	0	<1
6	AER.3.1	*Aeromonas allossacharophila*	Eau de rivière (Marne Saint Maur)	2,00E+04	0	<1
7	AER.3.2	*Aeromonas allossacharophila*	Eau de rivière (Marne Saint Maur)	1,00E+03	0	<1
8	AER.4.1	*Aeromonas veroni*	Eau de la Seine	1,50E+04	0	<1
9	ART.1.1	*Athrobacter spp*	Nébulisateur d'eau	2,00E+03	0	<1
10	ESC.1.111	*Escherichia coli*	Eau de fontaine	1,40E+04	0	<1
11	ESC.1.117	*Escherichia coli*	Eau de puits	2,60E+04	0	<1
12	PSE.2.1	*Pseudomonas fluorescens*	CIP 69.13T	2,10E+03	0	<1
13	PSE.2.2	*Pseudomonas fluorescens*	CIP102127	2,80E+03	0	<1
14	PSE.2.3	*Pseudomonas fluorescens*	INRA PF 2-495	5,60E+03	0	<1
15	PSE.3.1	*Pseudomonas putida*	Eau	7,30E+03	0	<1
16	PSE.4.1	*Pseudomonas pseudoalcaligenes*	Eau	1,70E+04	0	<1
17	RAH.1.1	*Rahnella aquatilis*	CIP 78.65	4,20E+03	0	<1
18	STE.1.1	*Stenotrophomonas maltophilia*	Eau de fontaine	8,80E+04	0	<1
19	XAN.1.1	*Xanthomonas campestris*	Evaporateur de climatisation automobile	2,40E+04	0	<1
20	AUR.1.1	*Aureobacterium saperdae*	Evaporateur de climatisation automobile	1,30E+04	0	<1
21	EWI.1.1	*Ewingella americana*	Environnement aquatique	3,80E+04	0	<1
22	RAH.1.2	*Rahnella aquatilis*	Eau (Seine)	4,40E+04	0	<1
23	MOR.1.1	*Morganella morganii*	Eau de rivière (Bièvre)	6,30E+04	0	<1
24	VIB.3.1	*Vibrio aglinolyticus*	Eau	1,40E+04	0	<1
25	SER.3.2	*Serratia marcescens*	Eau	9,00E+04	0	<1
26	ENTC.1.4	*Enterococcus faecalis*	Eau de rivière (Tamise)	7,40E+04	0	<1
27	AERC.1.1	*Aerococcus viridans*	CIP 54.145	1,10E+04	0	<1
28	AER.5.5	*Aeromonas media*	Non treated water	2,80E+03	0	<1
29	AER.1.8	*Aeromonas hydrophila*	Non treated water	2,50E+04	0	<1
30	AER.6.1	*Aeromonas caviae / enteropelogenes*	Non treated water	8,00E+04	0	<1
31	PSE.5.1	*Pseudomonas monteilii*	Non treated water	5,10E+04	0	<1
32	AER.5.1	*Aeromonas media*	Non treated water	2,10E+04	0	<1
33	PSE.1.12	*Pseudomonas ~~aeruginosa~~ monteilii*	Lake water	38	0	<1
				107	0	<1

Appendix 8: interlaboratory study raw data

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
F	1	54	54	42	88.5
	2	0	<1	0	<1
	3	66	66	43	94.5
	4	25	25	28	40.6
	5	46	46	36	62.4
	6	54	54	37	65.9
	7	21	21	27	38.4
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
G	1	72	72	46	118.4
	2	0	<1	0	<1
	3	31	31	36	62.4
	4	15	15	28	40.6
	5	27	27	38	69.7
	6	25	25	37	65.9
	7	23	23	21	27.1
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
H	1	62	62	29	42.9
	2	0	<1	0	<1
	3	34	34	38	69.7
	4	25	25	25	34.4
	5	41	41	31	47.8
	6	44	44	37	65.9
	7	24	24	22	28.8
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
I	1	79	79	0	<1
	2	0	<1	0	<1
	3	84	84	0	<1
	4	38	38	0	<1
	5	54	54	0	<1
	6	58	58	0	<1
	7	28	28	0	<1
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
J	1	70	70	33	53.1
	2	0	<1	0	<1
	3	87	87	33	53.1
	4	41	41	22	28.8
	5	57	57	22	28.8
	6	52	52	28	40.6
	7	55	55	12	13.7
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
K	1	51	51	38	69.7
	2	0	<1	0	<1
	3	57	57	35	59.1
	4	15	15	18	22.2
	5	29	29	25	34.4
	6	36	36	26	36.4
	7	28	28	29	42.9
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
L	1	32	32	39	73.8
	2	0	<1	0	<1
	3	41	41	39	73.8
	4	13	13	27	38.4
	5	25	25	35	59.1
	6	32	32	34	56.0
	7	13	13	22	28.8
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
M	1	71	71	36	62.4
	2	0	<1	0	<1
	3	73	73	49	165.2
	4	30	30	25	34.4
	5	50	50	31	47.8
	6	49	49	29	42.9
	7	39	39	27	38.4
	8	0	<1	0	<1

Laboratory	Sample	Reference method: NF EN ISO 16266: 2008		Alternative method: Pseudalert	
		Number of typical colonies (blue/green)	Final result in CFU/100 mL	Number of fluorescent wells	Final result in MPN/100 mL
N	1	93	93	31	47.8
	2	0	<1	0	<1
	3	69	69	37	65.9
	4	37	37	21	27.1
	5	64	64	28	40.6
	6	69	69	32	50.4
	7	45	45	24	32.4
	8	0	<1	0	<1

Appendix 9: Enumeration of culturable microorganisms

Laboratory	Culturable microorganisms at 22°C	Culturable microorganisms at 36°C
A	<1	<1
B	Vial to the enumerations of culturable microorganisms broken	
C	<1	<1
D	<1	<1
E	2	2
F	<1	<1
G	Confluent grow	Confluent grow
H	<1	<1
I	3	1
J	<1	<1
K	<1	<1
L	<1	<1
M	1	2
N	<1	7
Expert	2	3